Estimation of soil organic carbon stock by fixed depth and equivalent soil mass methods in fine and coarse textured soils with cereal and grass based cropping history

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Master's thesis

Department of Agricultural Science

Environmental Soil Science

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Abstract
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Abstract

The agricultural management of soil organic carbon (SOC) is highly important to build a climate-smart and eco-friendly agricultural system. For this reason, our study was conducted to test the hypothesis that the effect of different treatments consisting of soil textures and crop varieties on SOC stock has a major influence on any loss or accumulation of SOC storage. We also test the hypothesis that equivalent soil mass (ESM) is more consistent than fixed depth (FD) in estimating SOC content. In our study, the results were not found statistically significant but they showed minor differences in the SOC estimation. The difference was not clearly observed between ESM and FD methods. The influence of soil textures and crop varieties on SOC content was not significantly different. However, the cultivation of grass in fine-textured soils slightly increased the SOC stock compared with that in coarse-textured soils probably due to enhanced grass root growth and aggregation in fine textured soils that protects soil organic matter from microbial oxidation.

Keywords

Soil organic matter (SOM), soil organic carbon (SOC), Fixed depth method, aggregation, Equivalent soil mass method, carbon sequestration.

Where deposited

Environmental Soil Science Unit, Department of Agricultural Sciences

Further information

Supervisors: Dr. Asko Simojoki, Dr. Sanna Kanerva, Dr. Jussi Heinonsalo

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1 Introduction

The amount of carbon (C) stored in soil is equivalent to the amount stored in both the atmosphere and terrestrial vegetation (Conant et al. 2003). Soil organic carbon (SOC) is an essential component of soil quality as it improves soil structure, nutrient cycling and water holding capacity (Han et al. 2010). However, the contribution of soil organic carbon to the emissions of carbon dioxide (CO_2) is a global concern since CO_2 is one of the most important greenhouse gases.

Soil is the largest reservoir of organic carbon in the terrestrial biosphere (biota and their surroundings) and even small losses of this carbon pool can cause huge increase in the amount of CO_2 concentration in the atmosphere (Johnston et al. 2004; Xu et al. 2011). Consequently, soil carbon loss has a significant impact on global carbon cycle, global warming and climate change. Hence, the sequestration of carbon into the soil from atmosphere is important to maintain the balance of SOC between soil and atmosphere. Co-benefits of soil C sequestration include: advancing food and nutritional security, increasing renewability and quality of water, improving biodiversity and strengthening elemental recycling (Lal et al. 2015).

The extensive changes in land uses due to deforestation, biomass burning, tillage, drainage and off-farm fertilization substantially affect the SOC pools (Lal 2003). Besides, climate, land cover, soil order and soil texture influence the soil organic carbon pool (Batjes 1998). In addition, the land use changes and agricultural management practices leading to mineralization, leaching and erosion contribute to about 50-70% loss of SOC as CO_2. During 2007-2016, the changes in land use, agricultural activities and forestry has contributed to about 13% of CO_2 emissions globally (Mbow et al. 2017). Consequently, the cropping practices and soil particle sizes highly influence the amount and net balance of SOC in the soil profile. It's also known that, SOC is better retained belowground by the addition of plant inputs and organic carbon than carbon from aboveground plant parts (Rasse et al. 2005). While, many previous research was confined to the top soil carbon measurement up to 20 cm depth, the amount of SOC globally, has been estimated to be 1500 and 2300 Pg between 1 and 3m depth (Jobbágy & Jackson 2000; Yu et al. 2010). For these reasons, it is highly important to have more knowledge about the impact of different cultivation practices on SOC pool and its distribution in the soil profile in order to develop a climate-friendly agriculture.

The estimation of the magnitude of soil organic carbon pool is important for assessing the contribution of soil in global carbon cycle (Yang et. 2007). The result of soil organic carbon assessment varies in different methods. Fixed depth method is commonly used in SOC estimation. However, the fixed depth method may cause a significant errors in quantifying SOC stock due to the difference of bulk densities originating from agricultural management practices (Ellert and Bettany 1995; Ellert et al.

2002). The SOC amount can be more accurately estimated by using the equivalent soil mass method (ESM) (Ellert and Bettany 1995; Gifford and Roderick 2003).

In this master's thesis, the profiles of fine and coarse textured soils under cereal and grass cropping were studied to 1 meter depth to better understand their carbon sequestration. Two different methods, fixed depth (FD) and equivalent soil mass (ESM) method were used to investigate the amount of SOC content. This research was done as a part of research cooperation for Carbon Action Project in collaboration with Dr. Jussi Heinonsalo. This project was introduced in 2017 by Baltic Sea Action Group (BSAG) and Finnish Meteorological Institute. The project aims to build a climate-smart agriculture and sustainable practices for the farmers.

2 Literature review

2.1 Soil organic matter (SOM)

2.1.1 Definitions and chemical nature

Soil organic matter consists of identifiable plants and animals parts that have been transformed to a degree that it does not anymore remain at its original structural arrangement (Oades 1989). According to the progressive decomposition model, soil organic matter (SOM) is composed of a range of organic fragments and all sizes microbial products at multistage of decomposition while soil continuum model concept defines, that soil organic matter is a continuum of organic fragments that are progressively decomposed by microbial communities to result in smaller molecular organic compounds (Lehmann and Kleber 2015). The unidentifiable materials are termed as humus which in majority soils make up the most of soil organic matter. According to Waksman (1936), humus is defined as brown to dark colored complex aggregates of amorphous and colloidal substances evolving during decomposition of plant and animal residues by microbes under aerobic and anaerobic conditions (Senn and Kingman 1973).

Photosynthesis by plants is the main process to produce soil organic matter and so to soil organic carbon. In this process, atmospheric carbon dioxide and water react to synthesize organic products with the help of light energy. The basic reaction of photosynthesis is presented below;

$$6\ CO_2 + 6\ H_2O + \text{Light energy} = C_6H_{12}O_6 + 6\ O_2$$

Naturally occurring agricultural flora and fauna may contribute to around 609 Pg soil organic carbon (SOC) to 1 m depth (Stockmann et al. 2013). The flora, fauna and microbial communities that undergo multi stages of decomposition are key to the accumulation of soil organic carbon (Kleber and Johnson 2010). The compounds derived from SOM have varied turn over time and rate since they are affected by complex physical, chemical, and biological interactions and processes in the soil (Post and Kwon 2000). Plant litters and the originating biomacromolecules can be intrinsically refractory due to the presence of lignin, tannin, cutin and suberin (Figure 1), while for example sugars, starches and simple proteins are easy and quick to decompose (Brady et al. 2008). Recalcitrance can also be the result of chemical condensation and complexation processes occurred during decomposition stages which contribute to building and achieving stable aromatic and long aliphatic chain compounds (Paul et al. 1997). This is highly important for soil carbon sequestration (Derenne and Largeau 2001).

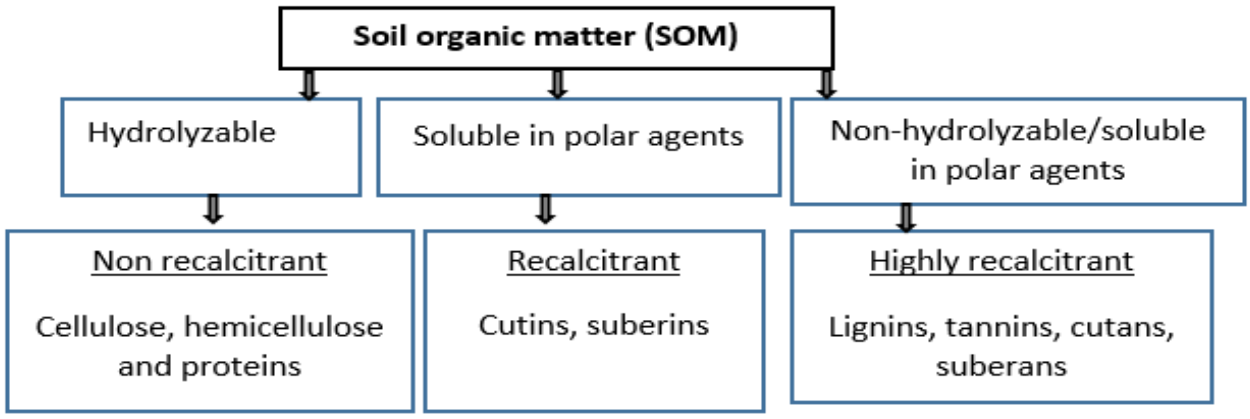


Figure 1. Different type of biomacromolecules derived from soil organic matter with their chemical nature (Adapted from Lorenz and Lal 2005).

2.1.2 Transformation processes of SOM

The physical and chemical or biological breakdown of dead organic matter by which the evolution of carbon dioxide takes place and nutrients are released is called decomposition (Chapin III et al. 2011). In the first steps of decomposition, the enzymatic breakdown converts the larger molecules including poly-aromatics, proteins and lipids into their monomers having smaller and simpler structure like carboxylic acids and amino acids by depolymerization and oxidative reactions (Lehninger et al. 2005). The second steps involves decomposition of these molecules by soil heterotrophic organisms that lead to releasing inorganic substances such as nutrients, water and CO_2. This microbial decomposition to convert organic substances to water soluble inorganic form is known as mineralization and it is one of the decomposition processes (Brady et al. 2008).

2.1.3 Soil organic carbon and its importance

The term soil organic carbon is commonly defined to indicate the carbon component of the soil organic matter. It is often used for the quantitative measurement of soil organic matter. About half of the soil organic matter is soil organic carbon by weight. Therefore, soil organic matter is usually estimated as two times of soil organic C (SOM=2*SOC) (Brady et al. 2008). Soil organic carbon can be found in particulate organic matter (POM), which is mostly a plant origin particulates of 0.053 to 2mm in size while mineral associated organic matter (MOM) is predominantly microbial products. Mineral associated organic matter is composed of single or microscopic sizes molecules of organic material deriving from plants or resulting from the chemical changes by soil microbes. The added organic materials containing organic carbon increase the carbon storage in soil and hence may reduce the greenhouse gas emissions including N_2O while improving the soil structure (Mutegi et al. 2010;

Hu et al. 2018). A proper management of soil organic matter enhances SOC content and soil nutrient storage by increasing the level of nitrogen, phosphorus, sulphur and save soils from draught by enhancing soil hydraulic properties (Kirkby et al. 2011; Gomiero et al. 2011).

2.1.4 Pools of SOC

The common pools of soil organic carbon are active or labile pool and passive or stabile pool. Both have significant role in SOC dynamics and nutrient cycling.

The labile pool of soil organic carbon is the dynamic fraction of soil organic carbon and it is influenced highly by disturbances and soil management practices to result in the highest turnover rates (Coleman and Crossley 1996). Its average turnover time is less than a few years while it is even several thousand years for recalcitrant carbon (Parton et al. 1987). This labile fraction of soil organic carbon is composed of amino acids, simple carbohydrates and other simple organic compounds and also a fraction of microbial biomass.

The stabile soil organic carbon is the fraction of SOC having slow turnover time between centuries to millennia. The short-term land management practices cause hardly any effect on this stable SOC pool (Wang and Hsieh 2002). As a result, it remains as a long term storage of SOC (Helfrich el al. 2007).

2.1.5 Dynamic sources and factors controlling soil organic carbon

The sources of soil organic carbon are decomposing plant materials, microbial residues and remaining of soil flora and fauna. With the accumulation of SOC in the soils, a fraction of it is lost through gaseous emission of CO_2 (Figure 2). The main source of SOC is plant litter while microbial residues act as the secondary source (Krull et al. 2003). Another source of SOC in the soil is rhizodeposition. It includes the input of organic carbon compounds from living roots, root exudates, root caps and it is responsible for noticeable changes in soil biological, chemical and physical properties. For instance, rhizodeposition as a source of low molecular weight organic substances (Dennis et al. 2010) highly boosts the soil biological activity in the rhizosphere. (Nguyen 2003; Bais et al. 2006).

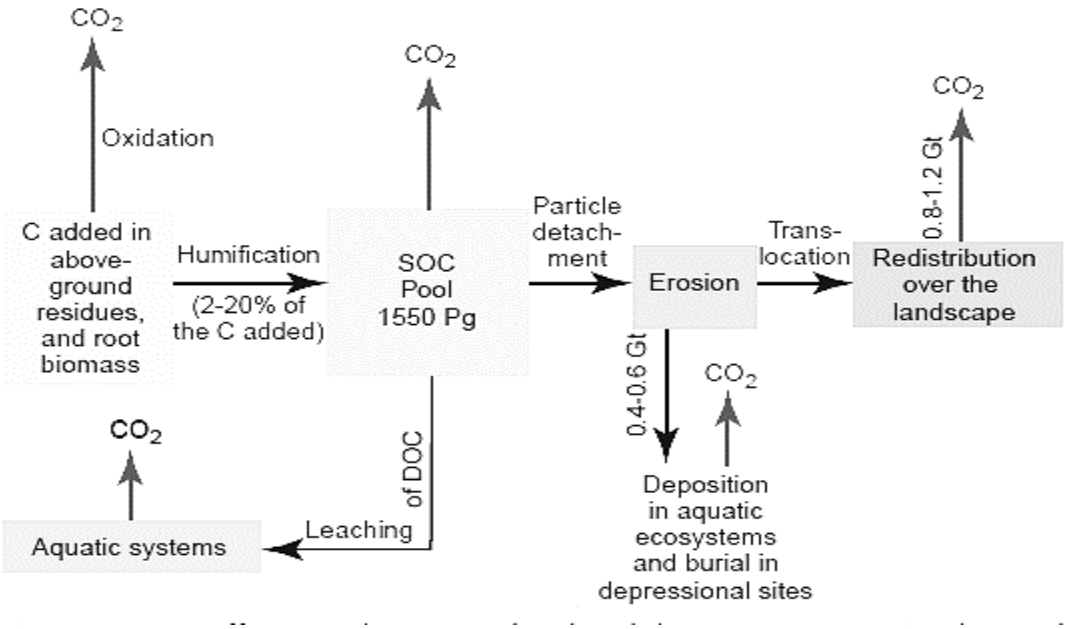


Figure 2. Processes involved in SOC dynamics (Adapted from Lal 2004).

The interrelated major driving forces controlling the SOC dynamics include climatic factors (precipitation and temperature), soil conditions with different physico-chemical characteristics such as mineralogy and biotic factors that are mainly responsible for the amount and quality of the carbon deposition into the soil (Luo et al. 2017). In addition, anthropogenic factors such as land use, fire, tillage, N deposition and other management practices affect SOC dynamics (Jackson et al. 2017).

2.2 Stabilization of SOM for SOC sequestration

Various stabilization and destabilization processes regulate the occurrence, distribution and deposition of soil organic matter. Stabilization of soil organic matter is defined as the decrease in the potential loss of SOM by different processes including respiration, erosion or leaching (Sollins et al. 1996). Stabilization of SOM and the evolving soil organic carbon can be achieved by soil aggregation, organo-mineral complexes and by enhancing biochemical recalcitrance. For instance, cutins and suberin are selectively preserved in POM due to its inherent recalcitrance or in mineral associated organic fractions because of preferred association with mineral surfaces (Rumpel et al. 2004; Filley et al. 2008). Some of the stabilization mechanisms are discussed below;

2.2.1 Aggregation and SOC binding agents

Soil aggregates are naturally occurring cluster of particles that are held together more strongly than the adjacent particles (Nweke & Nnabude 2014). According to Tisdall and Oades (1982), microaggregates consists of primary particles and silt-sized aggregates (< 20 μm) with size ranging between (20-250 μm) and these stable microaggregates later form macroaggegates having sizes > 250

μm. An improved soil aggregation protects soil organic carbon within aggregates (Amado et al. 2006), because, aggregates prevents the oxidation of SOC to CO_2 through microbial activity.

There are three types of organic binding substances including temporary, transient and persistent which are responsible for the stabilization and configuration of soil aggregates (Tisdall and Oades 1982). The different temporary agents are bacterial cells, algae, plant roots, fungal and mycorrhizal hypae which are full of carbonaceous elements. The clay particles are accumulated and adsorbed by these different agents. These in turn help in the formation and stabilization of macroaggegates (Cambardella and Elliott 1993). The organo-mineral complexes because of their persistent nature stabilizes the maccroaggregates for long-term (Greenland and Fertilizers 1965).

Polysaccharides and organic mucilage's constitute the transient binding agents. Polysaccharides are easily released from organic residues and decomposed rapidly by microbial actions. The sources of these polysaccharides are plant and animal tissues and root exudates, bacterial cells, fungal hyphae and plant roots (Tisdall and Oades 1982). Though polysaccharides shares 25% of the humus but they are mostly important materials for aggregate formation (Chenu and Guerif 1991). Polysaccharides bind aggregates through polymer bridges and are generally help in binding 10-50 μm sizes aggregates. Polysaccharides, being relatively less mobile and negatively charged interact with clay particles which increases the inter-particle's bonding strength (Chenu and Guerif 1991). However, due to rapid decomposition, the stabilizing time of aggregates influenced by polysaccharides commonly lasts for a few weeks.

The persistent binding agents are recalcitrant form of organic materials that include humic compounds, polymers and polyvalent cations. Due to high molecular weight, the humic compounds are chemically recalcitrant and these agents are linked with micro aggregates formation and SOC sequestration. Although, they have a long term effect on micro aggregation processes but the longevity of carbon in micro aggregates influenced by persistent agents is yet to be fully clear (Gale et al. 2000).

2.2.2 Mechanism of organo-mineral complex formation

Clay minerals having high surface area are able to adsorb much SOM while protect it from microbial enzymes. The electrostatic forces favors the adsorption between organic molecules and clay minerals by strong bonds that in turn help the formation of organo-mineral complexes (Emerson 1959;Christensen and soils 1996). Carbon rich humic colloids as a source of organic anions and polysaccharides are adsorbed by clay particles and make bonds by polyvalent metal ions (Ca^{+2}, Al^{+3}, and Fe^{+3}) following a basic microstructure (clay particle-polyvalent metal-organic matter). Thus,

clay-humic complexes are formed through the chelation of carboxylic and hydroxide groups with the help of polyvalent cations (Ca, Al, and Fe). These complex interactions develop good soil structure and affect SOC dynamics**.** SOC encourages aggregation and in turn soil aggregates store SOC (Figure 3) by reducing the decomposition rate of SOM**.** The strong bonds help prevent the organic matter from microbial decomposition. The valence of the metal (Al^{+3}, Fe^{+3}, Ca^{+2}, Na^{+}) cations and the bonding types including H-bonding, Van der Waal's forces and columbic attractions determine the strength of the bonds (Greenland and Fertilizers 1965). Thereby, it requires high energy to break the bond and expose SOM for decomposition.

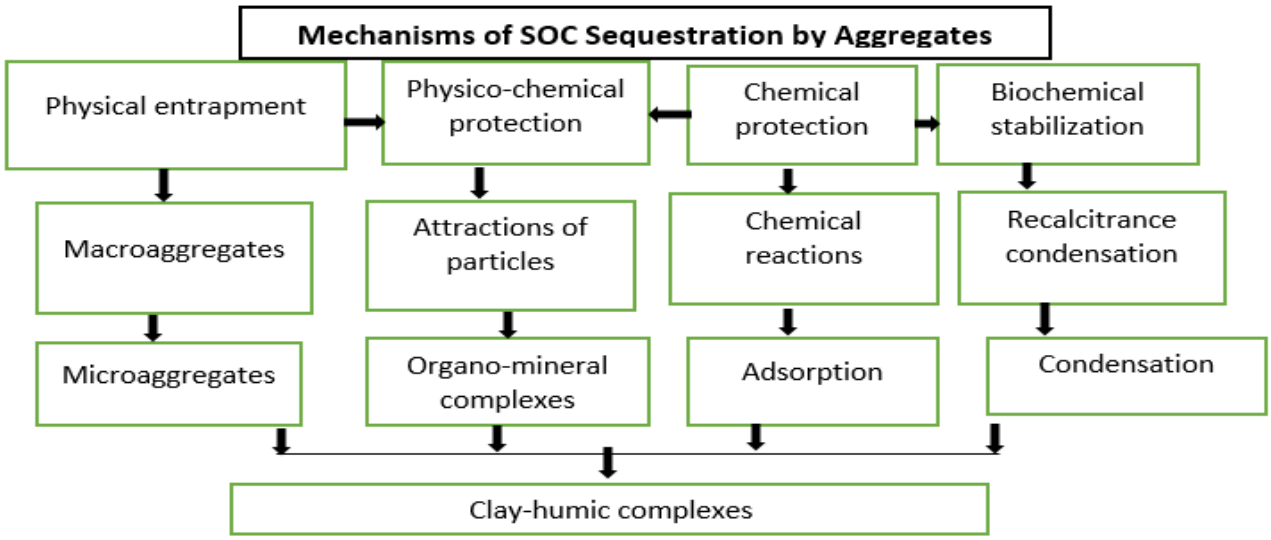


Figure 3. Steps involved in SOC sequestration by aggregates (Adapted from Emerson 1959; Tisdall and Oades 1982; Six et al. 2002).

2.3 Effect of soil texture on carbon storage

Soil texture influences the quantity of SOC (McConkey et al. 2003). The soils with higher clay content have a higher potential of holding soil organic carbon (Baldock and Skjemstad 2000). The additions of crop residues are decomposed more rapidly in sandy textured soils than in clay textured soils (McConkey et al. 2003). Clay soils are capable of retaining more SOC than sandy soils (Jenkinson 1988) because clay particles has many active sites and hence they are capable of adsorbing organic molecules which are stabilized and protected. In addition, the high bonding affinities to SOC (Sposito et al. 1999), the presence of Al and Fe hydroxides and resulted surface charge density due to isomorphic substitution or pH-dependent charges enhance the reactive sites and thus adsorption capacities. The organic matter is also trapped by flocculation of clay particles (Baldock and Skjemstad 2000) and it can also be trapped between sheets of interlayer silicate clay minerals (Theng et al. 1986).

It is reported that in temperate arable soils, clay holds 50-75% of the total SOC, while the share of silt is 20-40% and that of sand is less than 10% SOC pool (Christensen 2001).

2.4 Effect of agricultural practices on SOM and SOC sequestration

2.4.1 Effect of agricultural practices

Any plants that are grown for edible seeds is defined as cereals. Globally, cereal crops are cultivated largely for human food and it supplies higher amount of food energy as carbohydrate than any other crops (Ioannis and Persefoni 2008). The major cereal crops cultivated worldwide are wheat, maize, rice, barley, oats and sorghum. Intensive production of these crops is considered to have a negative impact on soil quality and sustainable agricultural system. The cropping intensification causes erosion, soil compaction and most importantly the loss of soil organic carbon and therefore contribute to the degradation of soil fertility (Hedlund 2012). Additionally, loss of SOC acts as a major source of greenhouse gas emission in the land use system.

The global soil carbon stock in top 1 m depth in grassland is estimated at 343 Pg (Conant et al. 2017). Perennial grasses are considered as the important source of soil carbon pools (Post and Kwon 2000). The amount of SOC is higher in deeper depths in grass cultivation compared to in row crop cultivation mainly because of high return of root biomass (Liebig et al. 2005). Other study concluded that pastures have higher belowground organic carbon concentration than wheat because they have longer vegetation period (Kuzyakov et al. 2000). Due to slow decomposition of roots and enhanced life span in soil, grass cultivation increases SOC stock. (Puget and Drinkwater 2001). Besides, roots releasing organic compounds contribute to soil micro and macro aggregate formation and stability and hence SOC is more protected in grasslands than in highly disturbed and intensively tilled croplands (Bronick and Lal 2005). Sharrow and Ismail (2004) reported that around 90% of organic carbon in pasture cultivation is belowground carbon. Besides, the intimate association between soil and roots help increasing the longevity of carbon as stored within micro aggregates. Consequently, grasslands act as potential sinks by lowering the enrichment of atmospheric CO_2 (Acharya et al. 2012).

The differences in root biomass and litter composition among grass species lead to varied potentials in SOC sequestration (Hebeisen et al. 1997). For example, white clover (*Trifolium repens*) has more SOC sequestration capacity than perennial ryegrass (*Lolium perenne*). The sequestration of SOC in white clover is higher, since the improved residue quality and high root biomass help the rapid formation of macroaggregates which in turn protect the SOC within (Six et al. 2001). On the other hand, alfalfa (*Medicago sativa*) entraps more SOC by promoting soil aggregation better than orchard

grass (*Dactylis glomerata*) (Min et al. 2003). However, there is a scope of more research to understand the mechanisms of soil organic carbon storage under the pasture cultivation (Six et al. 2000).

Tillage as an agricultural practice influences the processes of SOC dynamics and sequestration capacity within soil aggregates through the effect of soil disturbances. The organo-mineral surfaces are exposed to decomposers by tillage when it breaks the aggregates. By altering the cultivation practices, agricultural soils can have a huge potential of soil carbon sequestration (Post and Kwon 2000). It is found that no tillage (NT) protects the SOM from decomposition and stabilizes aggregates besides increasing the SOC pool (Lal 2002). It is also reported that no tillage and reduced tillage increase amount macro-aggregation which are rich in carbon (Gale et al. 2000; Six et al. 2000). Organic residues held in microaggregates are protected from microbial and enzymatic activities and thus remain relatively undecomposed for long. Frey et al. (1999) found that no tillage has increased surface layers organic carbon in the agricultural land while the storage differs for the soil profiles with tillage. To sum up, the SOC storage can be improved by increasing the input of organic matter, by delaying the decomposition rate of organic matter, by placing organic matter deep into the soil either by adding more below ground plant biomass or by mixing of surface materials by animals.

2.6 Methods soil C stock determination

The size of soil carbon stock can be estimated by using different methods. However, there may be differences in the estimations obtained by different methods.

2.6.1 Fixed depth method (FD)

Fixed depth method (FD) is used to estimate SOC stock in many previous researches. When using FD method, soil carbon stock is calculated as the product of organic carbon content (C %), bulk density (g/cm^3) and the thickness (cm) of the soil layer (Ellert et al. 2002; Wendt and Hauser 2013). Nevertheless, FD method provides a significantly biased estimation of SOC stock due to changes in bulk density as affected by the treatments of land use management and over time (VandenBygaart and Angers 2006; Murty et al. 2002; Ellert et al. 2002). It is observed that soil bulk density (BD) can be reduced to 10% or more based on the soil types (Onstad et al. 1984). Besides, bulk density increases with compaction at depth and very compact sub soils or strongly indurated horizons may exceed 2.0 g/cm3 (Cresswell and Hamilton 2002). Due to variation and inaccuracy in measuring bulk density, fixed depth method systematically overestimates the soil organic carbon and total nitrogen (TN) storage (Tong et al. 2018).

2.6.2 Equivalent soil mass method (ESM)

Due to biased estimation of SOC stock by FD method, calculations based on equivalent soil mass is considered to reduce the inaccuracy as caused by land use changes. Therefore, equivalent soil mass (ESM) method should be used in order to quantify SOC stocks appropriately (Ellert and Bettany 1995; Lee et al. 2009; Wendt and Hauser 2013).The quantitative determination of total SOC pool in a soil profile requires knowledge of both soil carbon content and the mass of soil as a function of soil depth. The reference soil mass per unit area belonging to a selected layer is defined as equivalent soil mass and equivalent carbon mass is the mass of carbon stored in ESM (Ellert et al. 2001). Because of the differences in the amount and distribution of plant materials in the soil profile under different agricultural management, soil carbon concentration varies at different depth (Staricka et al. 1991; Angers et al. 1995). Bulk density influences the calculation of soil carbon stock especially at soil surface due to maximum management activities and presence of plant roots and residues. In equivalent soil mass procedure, soil mass and organic carbon content are determined at different depth layers and therefore, the separate determination of bulk densities are not needed. Therefore, the use of soil mass gives a consistent basis for soil carbon stock estimation. ESM considers soil mass layers as 0-1000, 1000-2000, 2000-3000 $Mgha^{-1}$. The soil mass in the given depth layer of 0-10, 10-20, 20-30 cm changes with the change in bulk density whereas soil mass is assumed to be fixed in a soil mass layer. As a result, the estimation and comparison of organic carbon is harmonious (Wendt and Hauser 2013).

2.6.3 Schematic comparison between fixed depth (FD) and equivalent soil mass method (ESM)

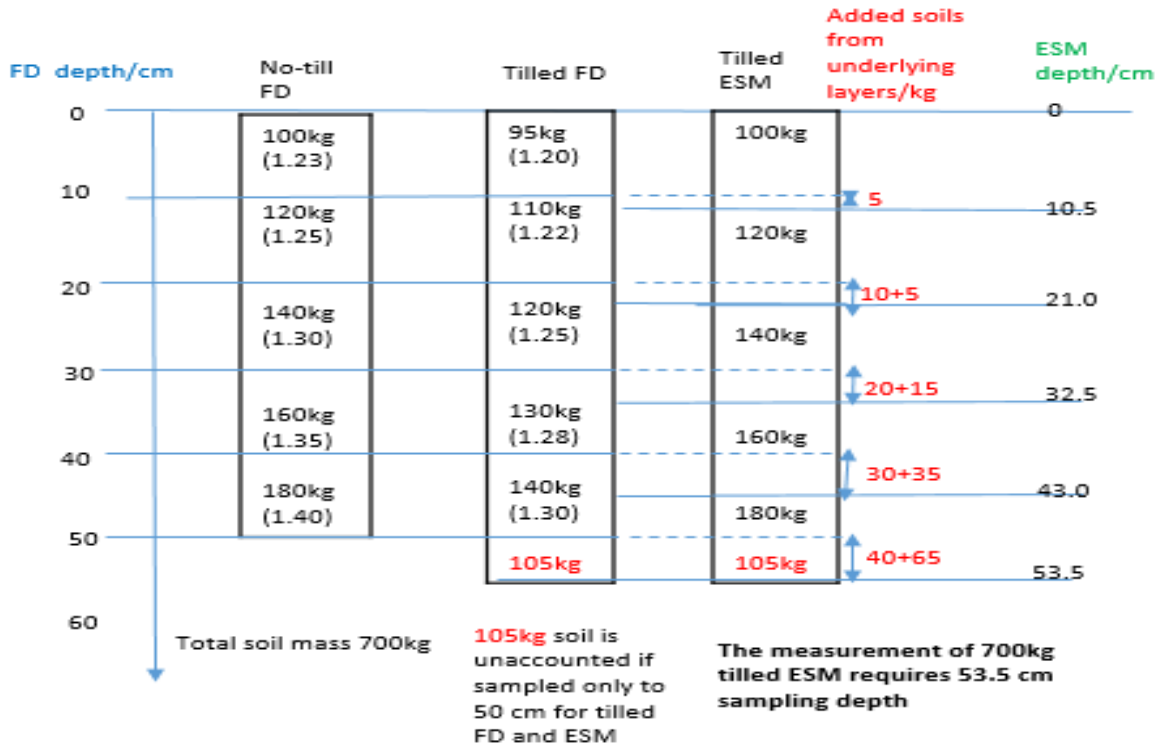


Figure 4: Hypothetical example of the equivalent soil mass (ESM) calculation in a tilled soil. The lateral dashed line indicates the fixed depth (FD) boundary and lateral solid line in tilled ESM column indicates the ESM boundary. Numbers in kg indicate the soil mass and numbers in the parenthesis indicate the bulk density (g/cm^3).

In tilled ESM, a portion of soil mass has to be added to the upper layers from the underlying layers to attain the corresponding equivalent soil mass. For example, in No-till FD (0-10 cm) contains soil mass of 100 kg whereas tilled soil contains 95 kg. As a result, 5 kg soil from the second layer was added to attain equivalent mass of 100 kg in the first layer. Consequently, after the subtraction, the soil mass in the second layer reduces to (110 kg-5 kg) =105 kg soil and thus, 15 kg soil from the third layer was added to attain equivalent soil mass of 120kg in the second layer. Similarly it goes to the subsequent layers which finally leaves a 105 kg of unaccounted soil mass. This 105 kg soil mass will be excluded if it is sampled to only 50 cm depth. For this reason, the ESM depth of 53.5 cm is required for obtaining same soil mass of 700 kg compared to 50 cm in No-till FD (Figure 4).

2.6.4 Effect of soil sampling depth

The shallow sampling to 20 cm in many previous researches underestimates the total carbon concentration and carbon pools in the soils. Consequently, the insufficient soil sampling provides results which give inconsistent conclusions in many studies of ecosystem services.

On the other hand, deeper sampling in other studies measured soil carbon stock below the topsoil and found the soil carbon content more than the amount in aboveground (Lorenz and Lal 2005; Homann et al. 2005; Nicoloso et al. 2009). Though, soil organic matter (SOM) is mostly concentrated to the top 30cm but deeper soil horizons have huge potential to sequester soil organic carbon due to slow decomposition and increased recalcitrance of soil organic matter (Liski and Westman 1995). Therefore, more information is needed to estimate SOC pool influenced by land use changes to at least 1 m depth. Thus, proper land use system including deep rooted crops and agricultural practices can sequester soil organic carbon into deeper layers through improving subsoil distribution and enhancing turn over time of SOC (Lal 2004; Post et al. 2004).

Previously, research was mainly confined to shallow sampling depth probably because research focused on shallow rooting crops and used cheap and easy sampling methods. Today, due to increased attention on soil carbon sequestration to deeper layers with deep rooting plants (such as Alfalfa), deep soil sampling is more common (Harrison et al. 2011). For this reason, sampling should include all mineral and organic soil layers to a depth of 1 m or even to the C horizons and the same sample should be used for measuring soil bulk density and soil carbon concentrations (Hamburg and Change 2000).

3 Research objectives

The main objectives of this thesis are to;

a. explain and compare the two main methods quantitatively (Fixed depth vs Equivalent soil mass) used for determining the distribution of SOC within soil profile.

b. observe the differences of SOC pool at different soil depths of fine and coarse textured soils under cereal and grass cultivation.

To fulfill the research objectives, this study aimed to estimating SOC stock by both fixed depth (FD) and equivalent soil mass methods (ESM) as influenced by soil textures and cropping history. These objectives were based on hypothesis that the equivalent soil mass is more consistent to estimate the SOC content than fixed depth method. It was also hypothesized that the combination of different soil textures and cropping may change the SOC pool differently under the agricultural management practices. The formation of soil structure and aggregation is more pronounced in fine textured soils than that of coarse soils. Therefore, soil organic carbon is trapped in the aggregates by fine textured soils that are thus assumed to have higher SOC storage than coarse textured soils. On the other hand, having higher root biomass and deeper rooting depth, grass cropping may contribute more to the SOC stock than cereal cropping practices. Besides, due to short vegetation period and high tillage frequency, the cultivation of cereal tends to decrease SOC contents compared to grass cropping practices.

4 Materials and Methods

4.1 Study sites and experimental setup

This study was conducted to examine the influence of previous cropping history on soil organic carbon in fine and coarse textured soils. The effects were tested in four treatments consisting of Coarse textured soil + cereal, Coarse textured soil + grass, Fine textured soil+ cereal and Fine textured soil + grass on SOC stock. Soil samples were taken from 12 farms in different parts of Western and Central Finland. The farms were located in the municipalities Parainen, Eura, Orivesi, Kiuruvesi, Lapua, Laukaa, Isokyrö, Urjala, Tammela and Keitele (Figure 5). There was one farm in each municipality except for two farms in both Laukaa and Urjala. The co-ordinate location of the municipalities are mentioned in order to better represent the sampling areas and they include Parainen (60.3011° N, 22.3022° E), Eura (61.1296° N, 22.1310° E), Orivesi (61.6774° N, 24.3574° E), Kiuruvesi (63.6538° N, 26.6211° E), Lapua (62.9720° N, 23.0024° E), Laukaa (60.1542° N, 23.0468° E), Isokyrö (62.9999° N, 22.3245° E), Urjala (61.0803° N, 23.5486° E), Tammela (60.8091° N, 23.7690° E) and Keitele (63.1785° N, 26.3412° E). The selected farms participated in the Carbon Action Projects[1]. The farms were selected with different soil textures and cropping history. According to USDA names, the coarse textured soils included glacial till soil with sandy loam texture (‘hietamoreeni’ according to Finnish textural class), sandy loam (‘karkea hieta’) and silt loam (‘hiesu’) soil while fine textured soils included silty clay loam soil (‘hiuesavi’) soils. For brevity, the fine and coarse textured soils are also referred to as fine and coarse soils, as well as clay and sand soils, respectively, in this thesis. The pH range of the coarse textured soils was 5.6-6.8 while the pH range in fine textured soils was 6.0-7.1. The amount of carbon in the coarse textured soils was in the range of 1.4-6.9% and in fine textured soils, it was 2.9-3.9%. Different crops were cultivated in the farms during 2014 to 2018. In the cereal cropping, the coarse textured soils were mainly cropped with sugar beet (*Beta vulgaris*), rye (*Secale cereale*), barley (*Hordeum vulgare*) and oats (*Avena sativa*) whereas beans (*Phaseolus vulgaris*), wheat (*Triticum aestivum*), oats and barley were grown in fine textured soils in those years. Similarly, other fields with the same soil textures under 5 years grass rotation were selected for the study. The grass species cultivated in the coarse textured soils were perennial grass (*Lolium perenne*), silage, red clover (*Trifolium pretense*) while pasture grass, silage and hays (*Phleum pratense*) were grown in fine textured soils. For brevity, the different cropping histories are also referred to as cereal and grass production/rotation in this thesis. The map in figure 5 shows the locations of soil sampling.

[1] (https://carbonaction.org/etusivu/)

Figure 5. Municipalities of soil sampling locations in this study (Adapted from Google map)[2].

4.2 Soil sampling and processing

In the fields of each of the 12 farms, three replicate sampling tubes with 1-m sampling depth were collected. A hydraulically driven mechanical auger attached to a tractor was operated to take the soil sample into a plastic tube. The tubes were capped and transported to the Viikki campus, University of Helsinki, where they were stored at $+4^0$ C until further processing. A total of 36 sampling tubes to be cut to a total of 360, 10-cm tube's pieces were expected to be processed and analyzed. In reality however, this total 36 sampling tubes provided a total of 261 tube's pieces containing equal amount of 261 soil slices which were processed for sample preparation and subsequent soil analysis in this study. The number of soil slices was assumed to be 10 slices in each 1 meter tube accounting for 360 tube's pieces but it reduced to 261 due to variability of the soil length inside the sampling tubes.

Before cutting of tubes, the surface soil was first made even by pressing it gently. Then, the surface depth of the soil inside the tube was measured and marked. The first 10-cm was marked on the tube surface by adding the surface depth value to the first 10-cm tube piece. Accordingly, the rest of the tube pieces were marked at 10-cm intervals. Then, the tube was cut using a special cutting device

[2] (https://www.google.com/maps/place/Finland/)

after adjusting the tube into marking areas. Each tube was cut down to its available sampling depth and or 100 cm at 10 cm intervals (0-10, 10-20, 20-30, 30-40, 40-50, 50-60, 60-70, 70-80, 80-90, and 90-100 cm. After cutting the tubes into 10 cm pieces, they were placed in a bag for further processing in the laboratory. An arrow was drawn on each piece of tube for indicating the natural direction of soil from top to bottom. During cutting the tubes, two persons cooperated; one person operated the machine after adjusting the marks on the tube, while other person helped in rotating the tube. After cutting the pieces, the plastic dusts was removed by a vacuum cleaner as plastic is also a source of carbon which can contaminate the soil sample.

4.3 Preparation of the soil sample

After cutting the tubes into pieces, they were stored in $\pm 4^0$ C cold room until further steps of preparation and analysis. Then, the whole tube pieces containing soil was weighed and noted down. Then, the soil was removed from the tube piece by pushing it upwards from below by a piston to get the soil slice. The resultant soil slice was cylindrical shaped. Then, the empty tube piece was cleaned and weighed. By subtracting the empty tube mass from the tube plus soil mass provided the fresh soil mass which was also noted down. Then, the cylindrical shaped soil slice was cut to about 0.5 cm margins from all sides by using a knife and or wire to get 1-2cm thick square shaped slice. This way, the contamination by the plastic dust and silicone contamination sprayed on the tube's wall was avoided. Later, the whole soil slice was cut in the middle from top to bottom and put in a bag in order to homogenize for different subsampling. From this portion of soil slice (approx. L 10-cm and H 1-2-cm), sample was taken for dry matter content determination and some microbiological analysis. Then, the rest of the slice (approx. L 10-cm and H 1-2-cm), was put in an aluminum box and make the slice into smaller separates (Figure 6). Subsequently, this aluminum box containing the soil slice (approx. L 10-cm, H 1-2-cm) was put inside a 30^0 C ventilated oven for 7 days until the weight was unchanged. The unchanged condition was checked by taking some representative samples. After completing air-drying, the soil parts were grounded in a mortar and then the soil was sieved by 2mm sieving. Then, the subsamples were taken for carbon and nitrogen analysis and also for other soil chemical analysis. The whole subsampling procedure is represented in Figure 6.

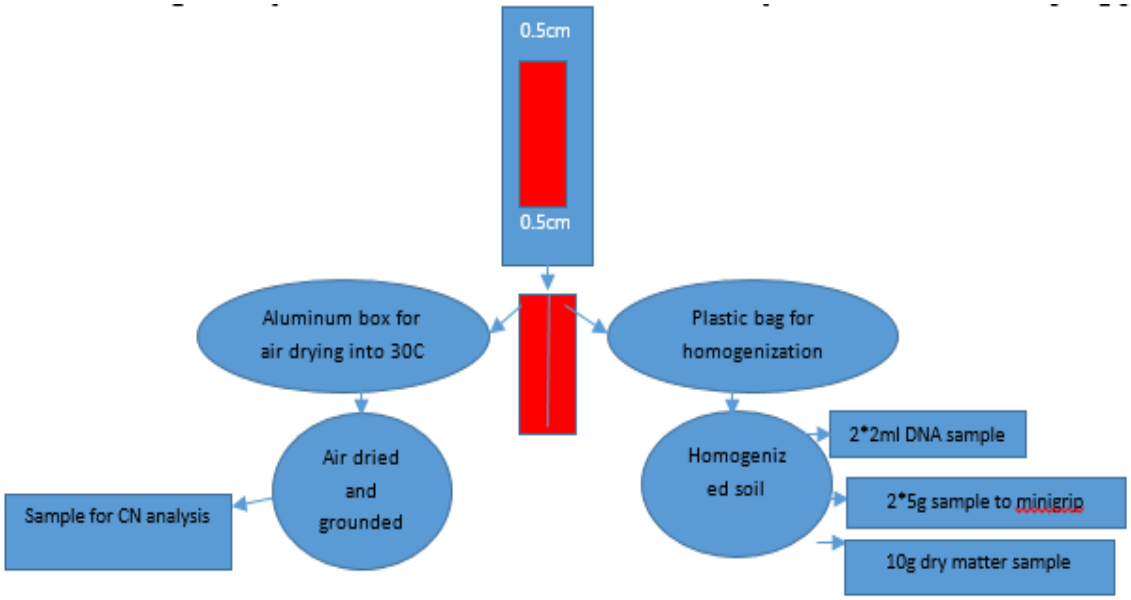


Figure 6. Preparation of sample at a glance

In this study, 10-g dry matter soil sample and air dried ground sample was taken for CN (carbon and nitrogen) analysis. The rest of the samples including 2-ml DNA sample taken into an Eppendorf tube and 5-g soil into a small minigrip plastic bag samples were placed inside a 3-L plastic bag and preserved at -20° C freezer for future microbiological and soil chemical analysis (Figure 6). Soil slices were processed and analyzed for collecting data in this study (Figure 7) as shown below;

Figures 7: Photos are showing different parts of data collection a) removal of soil slice by using piston b) soil slice output c) cutting soil slides by using wire d) separating middle portion of soil slice used for subsampling e) subsampling at a glance and f) data input. Source: Photos by author.

4.4 Measurement and analyses

About 10 g air-dry soil sample taken for CN analysis was finely ground with a mortar and pestle, and then sieved through a 2-mm mesh size. All the roots and other coarse materials larger than 2mm were removed after sieving. From this sieved and ground sample, around 300 mg subsample was taken in a foil paper that was inserted into the analyzer. Soil organic carbon was determined by dry combustion method by automated LECO CN analyzer (Model, CN 828), that reported the carbon and nitrogen percentages.

4.5 Calculation of soil C stocks

For the determination of soil dry matter content, a representative soil sample of about 10g was weighed fresh and also after drying in a ventilated oven at +105° C for 24 hours. The dry matter content of the soil sample was obtained by dividing the oven dried soil with the fresh soil mass (Equation 1). After determination of the dry matter content, the dry mass of soil ($m_{soil, dry}$) in the whole tube piece (10 cm) was calculated by multiplying its fresh wet mass with the subsample's dry matter content (Equation 7). The soil organic carbon was calculated by using following steps and different equations (2-9).

Procedures of raw data processing:

1. Dry matter content of fresh wet soil was obtained as DM_1 (weight of oven dried soil at 105° Celsius/ weight of fresh wet soil)

2. CN fresh wet soil sample was weighed before putting into 30° Celsius ventilated oven for air drying (W_1).

3. After, 7 days in 30° C oven drying, air dried soil was weighed (W_2)

4. Estimated air-dried soil mass was obtained as, $W_3 = (W_1 \times DM_1)$

5. Estimated dry matter content was obtained as $DM_2 = (W_3 / W_2)$

6. % C corrected = Leco % C/ DM_2

7. Dry soil mass ($m_{soil, dry}$) of the soil slice was attained as = Mass of fresh wet soil slice × DM_1

8. Mass of soil ($Mgha^{-1}$) was obtained as $M_{soil} = (m_{soil, dry} / Asoil) \times 10000 = (m_{soil, dry} / 3.1416 \times r^2) \times 10000$

9. Mass of OC ($Mgha^{-1}$) was obtained as $M_{oc\ (DL)}$ = Msoil × % C corrected.

Soil bulk density was calculated by dividing the dry mass of soil with the volume of soil. The volume of soil in the tube piece was obtained by measuring its diameter (d= 2r) and height (h) with a caliper scale. The cross-section of soil was assumed circular ($Asoil = 3.1416 \times r^2$). The bulk density was calculated as gram per cubic centimeter (g/cm^3). The bulk density (BD) and volume of soil (V_{soil}) in the tube piece were calculated by the following formulas:

$V_{soil} = (Asoil \times h) = (3.1416 \times r^2 \times h)$

$BD\ (g/cm^3) = m_{soil,\ dry}\ (g)/\ V_{soil}\ (cm^3)$

The fixed depth estimate of soil organic carbon content was calculated by the following equation:

$SOC\ (Mgha^{-1}) = Depth\ (m) \times Bulk\ density\ (Mg/m^3) \times C_{org} \times 10000$

The equivalent soil mass (ESM) estimate of organic soil carbon content was obtained as follows:

The mass of soil was calculated by dividing dry soil mass ($m_{soil,\ dry}$) with the cross section of soil (Asoil) (Equation 8). The total mass of organic C in each layer was obtained by multiplying the mass of soil (M_{soil}) with the mass of organic carbon (Equation 9). The total soil organic C stock at a given depth, and eventually in the whole soil profile, was finally obtained by summing up the amounts of C in the different soil layers in a cumulative fashion. Finally, the cumulative masses of dry soil and organic carbon (OC) as a function of soil depth were calculated by summing the values of individual layers above each given depth. A polyline interpolation was then used separately for each soil profile to estimate the OC content at any selected reference soil mass.

4.6 Statistical analysis

All statistical analyses were performed by using SPSS software (version 25.0). All data to 100 cm soil depth were used for boxplots of directly measured variables, whereas statistical tests of the effect of experimental treatments on SOC stocks at different soils depths and equivalent soil masses were carried out by using farm means of a given treatment as replicates. Due to missing samples at greater soil depths, the range of investigated soil depths had to be restricted to only 80 cm for statistical comparison of treatment means. A paired samples T-test with all data and farm means was conducted to check the differences in soil organic carbon (SOC) estimation by the fixed depth (FD) and equivalent soil mass (ESM) methods. Two-way ANOVA test was conducted to test the effects of soil texture and cropping history. This was followed by least significant difference (LSD) post-hoc test for the multiple comparison of treatment means. A multiple linear regression analysis was performed for soil depth, unfilled tube's gap (empty tube's part above the soil sample inside the tube) and organic carbon to predict the bulk density. All the differences were checked and discussed using the significance $p<0.05$ probability level, and tentatively at $p < 0.10$. Before performing all the analyses, the normality of the data was checked by using Shapiro-Wilk test. The data was normally distributed. Two samples including Ik 0.3 and Ni 0.2 were excluded from the analyses as they belonged to organic soils (>20% soil organic matter) while the other samples of this study were mineral soil samples.

5 Results

5.1 Range and other analyses of directly measured soil properties in the whole data set

Below the whole data set of 36 individual tubes was used for general description of selected variables in all layers of soil profile at 10-cm intervals in terms of boxplots. Then, factors affecting soil bulk density of sampled soil profiles were analyzed by multiple linear regression. The main difference of FD and ESM methods supporting the use of ESM method was illustrated by using data from "Ja" farm in this study. There were 4 treatments and each treatment consisted of 3 replicate farms when an individual replicate farm included 3 sampling tubes. The treatment effects were analyzed from the farm means.

5.1.1 Soil bulk density

The boxplot below in Figure (8) shows the ranges of variability and distribution of bulk density points in the topsoil and subsoil layers down to 100cm. In the topsoil (0-20 cm), the lowest point of bulk density was observed in treatment coarse + grass while the highest point and ranges of bulk density was found in coarse+cereal treatment (Figure 8a). It was observed that grass seemed to decrease bulk density in both soil textures. In case of subsoil below 20-cm, the lowest range of bulk density was observed in fine+grass followed by fine+cereal. The distribution of bulk density data varied much less in fine+grass treatment excluding the outliers than that of other treatment groups. Hence, this more consistent ranges of bulk density make the predictions more reliable in the subsoil layers. The treatment coarse+cereal has the highest median in the subsoil while coarse+grass treatment has the largest variability range of bulk density which is about (0.75-1.85). The outliers in the fine+grass treatment, indicated those bulk density observation fall outside the overall tendency of the distribution (Figure 8b).

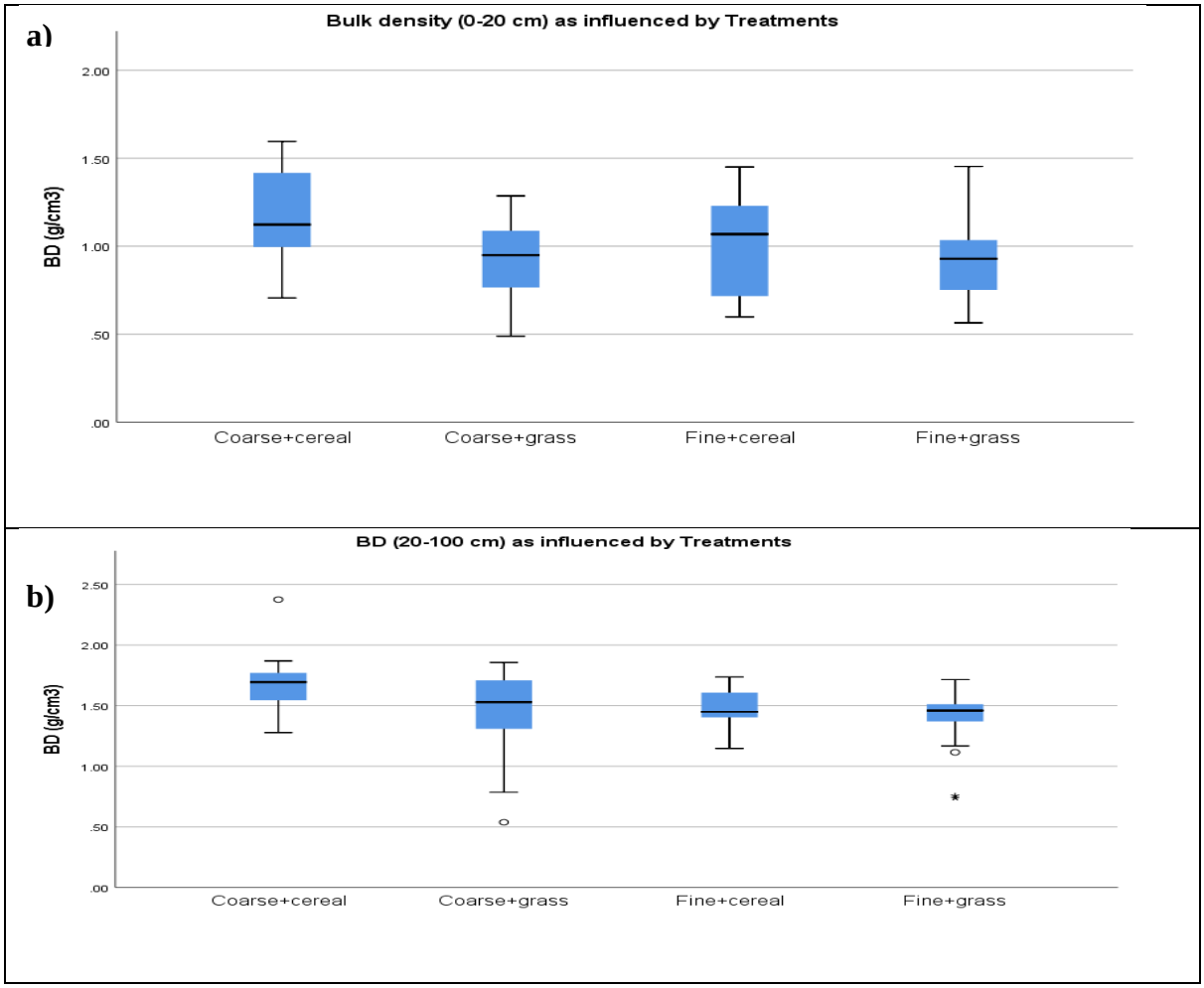


Figure 8. Boxplot of bulk density a) in the topsoil at 0-20 cm depth and b) in the subsoil at 20-100 cm depth in the different experimental treatments. The boxes are drawn between the lower quartile (Q1) and upper quartile (Q3). The boxes indicates the distribution of central 50% of bulk density data with middle line marking the median value. The whiskers are drawn to the nearest values not beyond the standard span (1.5 × (Q3-Q1)) from the quartiles. The outliers are indicated by small circles and asterisks.

5.1.2 Total mass of soil and organic carbon in the soil profiles

Figure 9a shows that the largest range of total soil mass in the soil profile (maximum cumulative soil mass) was found in the soil profile of fine+cereal treatment, while the variability was the smallest in fine+grass treatment (Figure 9a). In contrast the widest range of total soil organic carbon mass (cumulative SOC mass) corresponding to about 75-280 Mg (C) ha^{-1} was found in coarse+cereal which was followed by coarse+grass and fine+ grass treatments that covered much narrower ranges. The smallest ranges of SOC distribution in fine soils indicate a lower variability and oppositely a higher reliability than in coarse soils (Figure 9b).

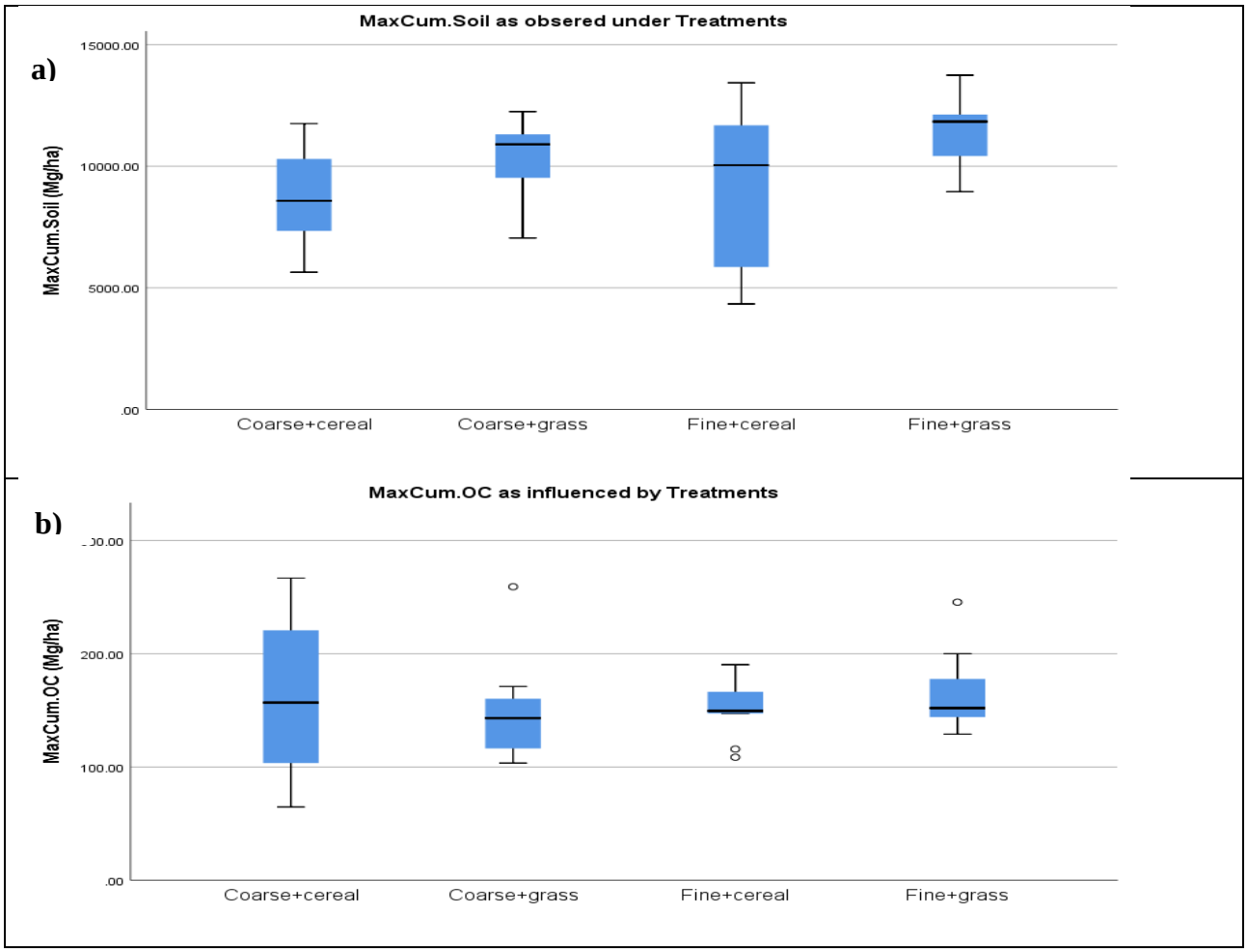


Figure 9. a) Total mass of soil (maximum cumulative soil mass, Mgha^{-1}) and b) total mass of organic carbon (maximum cumulative OC, Mgha^{-1}) in soil profile in the different experimental treatments. The boxes are drawn between the lower quartile (Q1) and upper quartile (Q3). The boxes indicates the distribution of central 50% of bulk density data with thick line marking the median value inside the boxes. The whiskers are drawn to the nearest values not beyond the standard span (1.5 × (Q3-Q1)) from the quartiles. The outliers are indicated by small circles.

5.1.3 Organic carbon content in topsoil and subsoil

According to figure 10a, the overall range of SOC stock in topsoil across all the treatments was about 8-57 Mgha^{-1} which also corresponds to coarse soils. On the other hand, the range in fine soils cropped with both cereal and grass was about 20-52 Mgha^{-1}. In the topsoil, cereal cropped fine soils had a higher median SOC stock about 37 (Mgha^{-1}) compared with the coarse textured soils (25 Mgha^{-1}). Besides, the lower quarter percentile in coarse textured soils indicates that 25% of the SOC distribution data was below the minimum SOC contents of fine textured soils. On the other hand, the SOC contents varied clearly less in fine textured soils compared with coarse textured soils under grass

cultivation (Figure 10a). In the subsoil below 20 cm, the overall range of SOC stock was found to be between 4-74 Mgha^{-1}. In between 20-100 cm, the SOC variability was greater in coarse+cereal treatment compared with all the other treatments. In cereal cropping, coarse soils showed the higher variability than fine soils. In contrast, coarse soils had higher SOC variability compared with fine textured soils under grass cropping (Figure 10b).

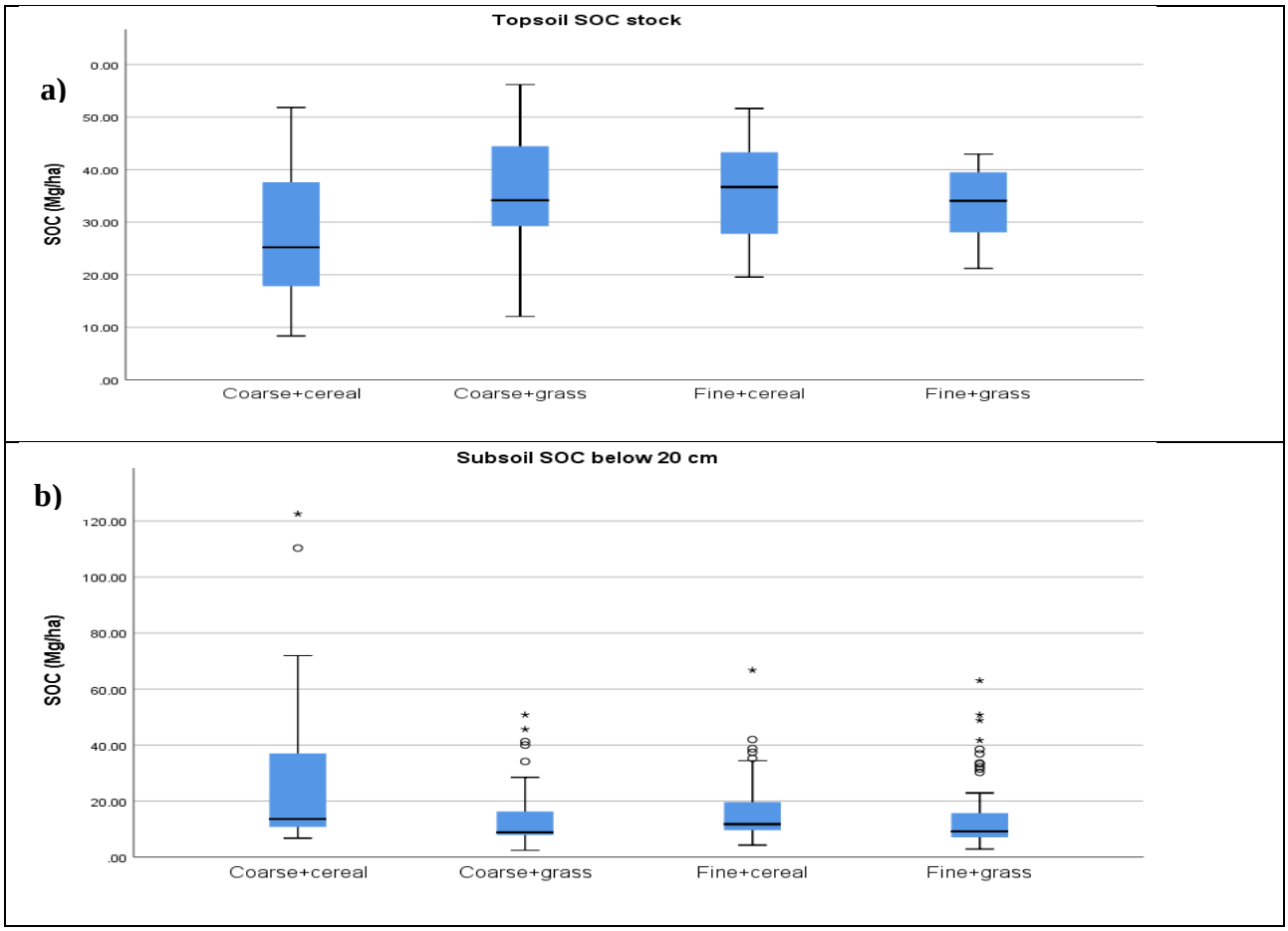


Figure 10. Boxplot of SOC stock a) topsoil (0-20 cm) and b) subsoil (20-100 cm) as influenced by treatments. The boxes are drawn between the lower quartile (Q1) and upper quartile (Q3). The boxes indicates the distribution of central 50% of bulk density data with thick line marking the median value inside the boxes. The whiskers are drawn to the nearest values not beyond the standard span (1.5 × (Q3-Q1)) from the quartiles. The circles and asterisks are indicating the outliers.

5.1.4 Factors affecting the bulk density of sampled soil profiles

As the FD estimation of SOC stock was based on soil bulk density, hence it might have been affected by any compaction of soil within the tube on sampling. Accordingly, the gap formation indicating partial filling due to possible compaction of soil tubes on sampling was common. Therefore, a multiple linear regression was conducted to investigate to which extent aimed soil sampling depth, observed gap in the tube after sampling (tube's gap as a surrogate variable for compaction) and

organic carbon content of soil could predict soil bulk density. The model predicted bulk density reasonably well (F 3, 244) = 67.366, p = < 0.01 with the predictor variables explaining 45.3% of the variation in BD. Soil depth and tube's gap increased the bulk density(regression coefficients B_{depth} = 0.003 and B_{gap} 0.005, p <0.01), whereas OC decreased BD (B_{OC} = -0.096, p <0.01). The final model was: BD (g/cm^3) = 1.256 + (0.003×Depth (cm) + (0.005×Gaps (cm) + (-0.096× OC %).

Table 1. Summary of multiple regression analysis for variables predicting bulk density (BD).

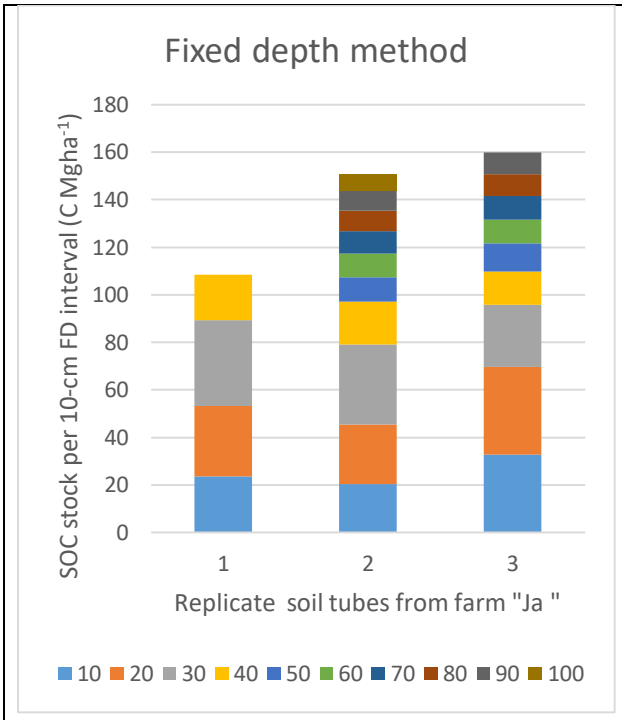
Variable	B	SE	ß	t	p
Constant	1.256	0.069		13.325	0.000
Aimed depth	0.003	0.001	0.267	3.921	0.000
Gaps	0.005	0.001	0.295	5.730	0.000
Organic carbon (OC)	-0.096	0.013	-0.472	-7.370	0.000

Dependent variable: Bulk density (BD)

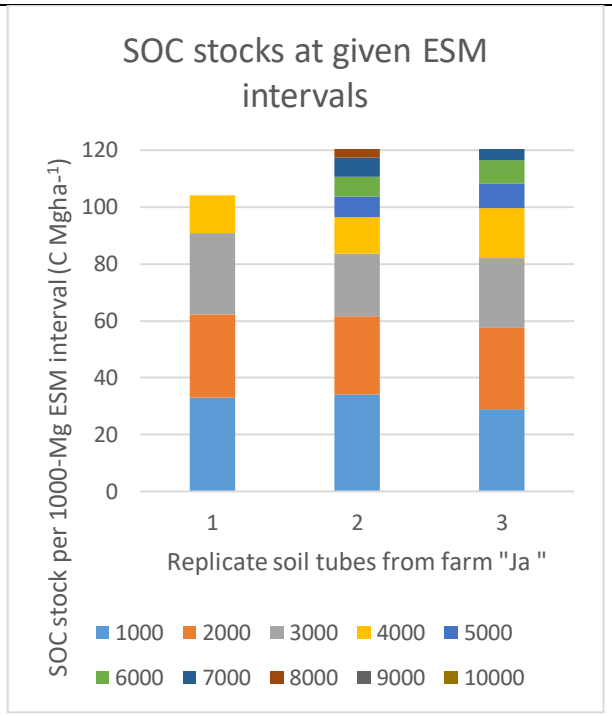
R^2 = 0.453

5.1.5 Illustration of within-field variation of SOC contents by FD and ESM methods

Figure 11 indicates that the estimates of topsoil SOC content within a single field of the farm "Ja" varied more by FD method compared with ESM method. This is especially the case for three uppermost soil layers. The effect of bulk density was more pronounced in the upper soil layers compared to subsoil layers.



a) FD variation in (Ja) farm



b) ESM variation in (Ja) farm

Figure 11. SOC content variability in three different replicate soil tubes from a single field of farm Ja determined by a) fixed depth (FD) method and b) equivalent soil mass (ESM) method.

5.2 SOC stocks in the soil profiles of the experimental treatments

The effect of the experimental treatments including combinations (soil type + cropping history) were investigated by using aggregated data of 12 farms. Three replicate farms represented each of the given four treatments in this study. In principle, the farm-means of estimated SOC stocks by FD and ESM methods were calculated as arithmetic means of the given layer in three individual soil tubes taken from the given field. However, sampling of deeper soil layers (below 40 cm) into the tubes was not always successful in all farms, which resulted in unequal number of replicates (n = 1-3) in different treatments at those depths.

5.2.1 Total SOC stocks as related to cumulative soil layer thickness in different treatments by FD method

Figure 12 shows that differences between the treatments were statistically not significant at 0-20, 0-40 and 0-60 cm. However, at 0-80 cm, coarse+cereal treatment had a significantly larger stock than the other treatments. The cumulative SOC stocks increased with soil depth in a broadly similar manner in each treatment. The main effect of soil layer depth across all treatments by ANOVA was significant and each depth differed significantly from each other ($p < 0.05$). The small inconsistencies

in the SOC accumulation with depth (such as in coarse soils under grass) are likely related to increasing number of missing replicates at soil depths below 40 cm (Figure 12).

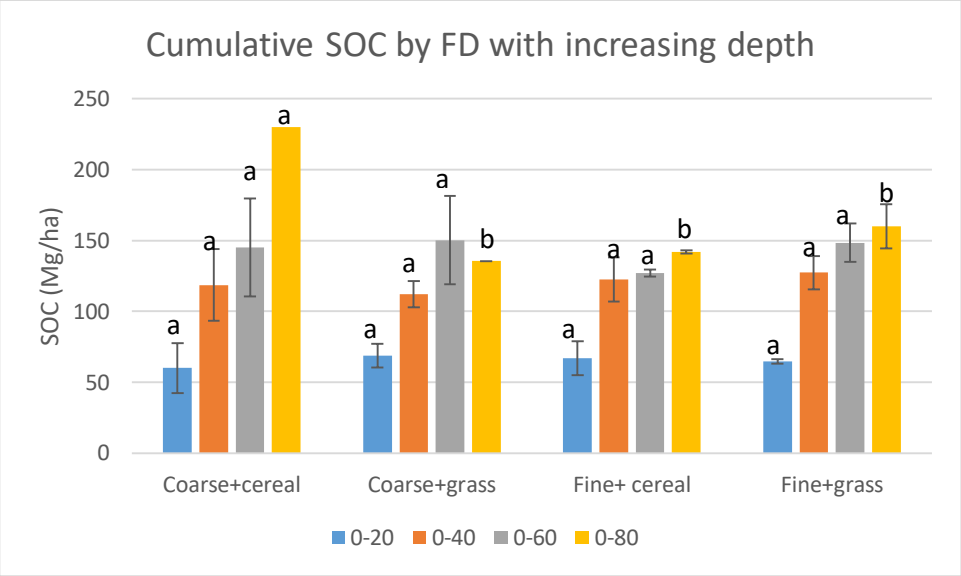


Figure 12. SOC distribution (Mean ± SEM) as related to soil depth in different experimental treatments. Significant differences among treatment means at a given FD level are denoted by different lower-case letters.

Mean SOC stock in soil layers of various depths, as well as ANOVA about the main effects and the interaction affecting them are shown in Table 2. The SOC-increasing effect of grass cultivation compared with cereal cropping in the topsoil and 0-60 cm level, as well as that of fine textured soil (clay) compared with coarse textured soils (sand) in top 40 cm turned out non-significant. Similarly, at shallow depths, the SOC storage tended to increase non-significantly with grass cropping and fine textured soils. In 0-80 cm layer, the main effects of soil and cropping history were statistically significant and there was also a significant interaction of soil type and cropping history at that depth. Unexpectedly, grass cropped soils had, on the average, a smaller SOC stock than cereal cropped soils, and clay soils had a smaller SOC stock than sand. However, the significant interaction means that the effects of cropping history were dependent on soil type. The significant interaction effect revealed that grass cropping had increased the SOC stock (relative to cereal cropping) more in clay soils than in sandy soils in the lower depths except the topsoil (Table 2).

Table 2. Effects of experimental factors soil texture (S) and crop (C) and their interaction (S×C) on SOC stocks at different FD levels.

Fixed level (Soil depth, cm)

SOC stock (Mgha^{-1})

Effect		**Mean:**	**0-20** **65.2**	**0-40** **120.2**	**0-60** **142.7**	**0-80** **166.8**
C_{grass}-C_{cereal}			3.2	-0.9	13.3	-38.3
S_{clay}-S_{sand}			1.6	9.6	-10.0	-31.6
($C_{grass}S_{clay}$-$C_{cereal}S_{clay}$)-($C_{grass}S_{sand}$-$C_{cereal}S_{sand}$)			-11.2	11.3	16.2	112.5
	MS_{error}		391.68	830.15	2008.70	358.32
	n		3	3	(2..) 3	(1..) 3
	SEM		11.43	16.64	25.88	10.93
		df		**p level**		
Crop	C	1	0.78	0.96	0.65	**0.06**
Soil	S	1	0.89	0.58	0.73	**0.09**
Crop ×Soil	C×S	1	0.64	0.74	0.78	**0.01****

The mean value indicates the average of all farms SOC stock (Mgha^{-1}) at a given FD level. C_{grass} and C_{cereal} treatment means of grass and cereal cropping treatments, respectively, across all fine soils (clay) and coarse soils (sand). S_{clay} and C_{sand} are treatment means of fine and coarse soils over all cropping histories, respectively. Their differences represents main effects of cropping and soil texture. Interaction effect ($C_{grass}S_{clay}$-$C_{cereal}S_{clay}$)-($C_{grass}S_{sand}$-$C_{cereal}S_{sand}$) shows how much larger the differences of grass and cereal cropping is in fine soils compared with coarse soils.

5.2.2 Total SOC stocks as related to cumulative soil layer thickness in different treatments by ESM method

According to Figure 13, the mean SOC stocks did not differ statistically significantly between any of the treatments. Numerically the top soil SOC content seemed slightly higher in coarse soils cropped with grass compared with all the other treatments. In the layers including 4000 and 6000, the SOC stock seemed largest in Fine+grass treatment, while at ESM level 8000 Mgha^{-1} the amount seemed largest in Coarse+cereal treatment (Figure 13).

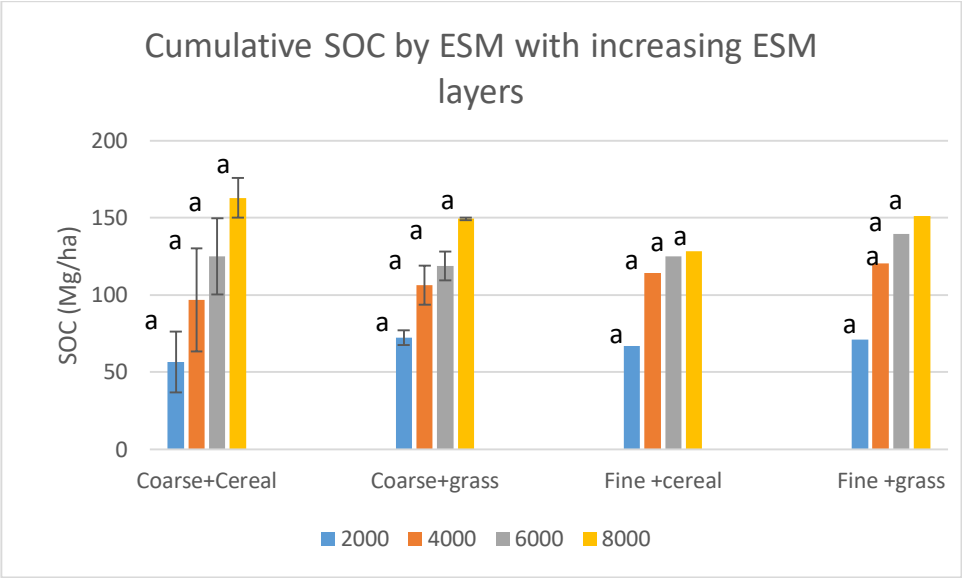


Figure 13. Distribution of soil organic carbon (SOC) ($Mgha^{-1}$) as related to ESM levels in different experimental treatments. Significant differences among different ESM levels in a given treatment are denoted by different lower-case letters. The treatment means at a given ESM level did not differ significantly ($p < 0.05$).

The effect of the cultivation of grass (relative to cereal cropping) increasing SOC stocks at all ESM levels and the effect of clay soils (relative to sandy soils) on increasing SOC stocks at ESM levels from 0 to 6000 $Mgha^{-1}$ turned out non-significant (Table 3). Grass and cereal cropping seemed to accumulate more SOC in fine textured (clay soils) than in coarse textured (sandy soils) in the deeper layers while the trend was opposite in the upper layers. However, the effect was not significant at any ESM level (Table 3).

Table 3. Effects of experimental factors crop (C) and soil (S) and their interaction (C×S) on SOC stock at different values of ESM soil mass.

Equivalent soil mass /$Mgha^{-1}$

SOC stock ($Mgha^{-1}$)

Effect		**Mean:**	**2000** **66.8**	**4000** **109.5**	**6000** **127.1**	**8000** **147.9**
C_{grass}-C_{cereal}			10.2	7.8	4.3	4.5
S_{clay}-S_{sand}			4.62	15.9	10.4	-16.4
($C_{grass}S_{clay}$-$C_{cereal}S_{clay}$)-($C_{grass}S_{sand}$-$C_{cereal}S_{sand}$)			-11.5	-3.2	21.2	36.2
	MS_{error}		421.04	1136.06	739.88	1006.98
	n		3	3	(2..) 3	(1..) 3
	SEM		11.85	19.46	15.71	18.32
		df		**p level**		
Crop	C	1	0.42	0.70	0.79	0.83
Soil	S	1	0.70	0.44	0.53	0.43
Crop ×Soil	C×S	1	0.64	0.94	0.52	0.38

The mean value indicates the average of all farms SOC stock ($Mgha^{-1}$) at a given ESM level. C_{grass} and C_{cereal} treatment means of grass and cereal cropping treatments, respectively, across all fine soils (clay) and coarse soils (sand). S_{clay} and C_{sand} are treatment means of fine and coarse soils over all cropping histories, respectively. Their differences represents main effects of cropping and soil texture. Interaction effect ($C_{grass}S_{clay}$-$C_{cereal}S_{clay}$)-(CgrassSsand-$C_{cereal}S_{sand}$) shows how much larger the differences of grass and cereal cropping is in fine soils compared with course soils.

5.2.3 Layer wise SOC stocks at different soil depths and ESM levels in experimental treatments by FD and ESM methods

Figure 14 shows that all the treatments are statistically non-significant at the depth level of 0-20 cm, 20-40 cm and 40-60 cm. However, at 60-80 cm, the treatments were statistically significantly different. The treatment coarse soils cropped with cereal were significantly different from rest of the treatments. The treatments coarse soil cropped with grass and fine soils cropped with both cereal and grass were found to be similar.

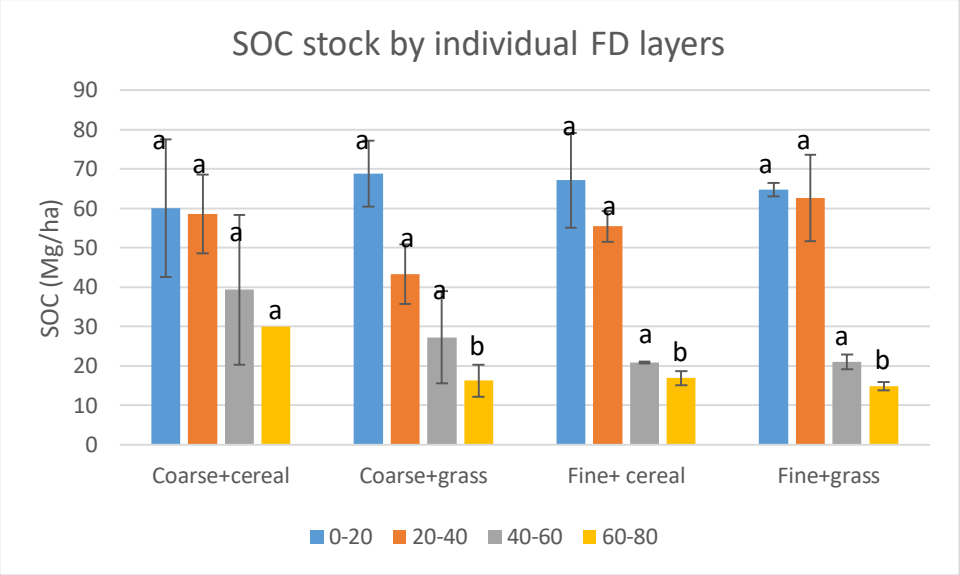


Figure 14. SOC distribution (Mean ± SEM) as related to individual soil depth in different experimental treatments. Significant differences among treatment means at a given FD layers are denoted by different lower-case letters.

The largest SOC stock was found in the topsoil while the mean value of SOC stock was found higher in the 20-40 cm layer which was almost double that in the layers 40-60 and 60-80 cm. The main effects of cropping and soil textures were found statistically significant at 60-80 cm, while the other FD levels showed a non-significant difference. It was noticed from the interaction that in relation to cereal cropping, grass cropping increased the SOC stock in fine clay soils about two-fold at 20-40 cm compared the effect in sandy soils. Unexpectedly, the SOC stock in clay soils was smaller than in sand in subsoil layers below 40 cm (significantly at 60-80 cm), and the SOC stock of grass cropped soil was higher in the topsoil while the difference was smaller compared with cereal cropped in other FD layers (significantly at 60-80 cm; Table 4).

Table 4. Effects of experimental factors C and S and their interaction (C×S) on SOC stocks at middle layers by FD method.

Fixed soil depth layers/cm

SOC stock (Mgha^{-1})

			0-20[3]	**20-40**	**40-60**	**60-80**
Effect		**Mean:**	**65.2**	**55.00**	**27.12**	**19.45**
C_{grass}-C_{cereal}			3.2	-4.0	-5.9	-7.9
S_{clay}-S_{sand}			1.6	8.1	-12.4	-7.3
($C_{grass}S_{clay}$-$C_{cereal}S_{clay}$)-($C_{grass}S_{sand}$-$C_{cereal}S_{sand}$)			-11.2	22.5	12.2	11.6
	MS_{error}		391.68	219.58	427.88	11.45
	n		3	3	(2..)3	(1..)3
	SEM		11.43	8.56	11.94	1.96
		df		**p level**		
Crop	C	1	0.78	0.65	0.65	**0.03***
Soil	S	1	0.89	0.38	0.36	**0.04***
Crop ×Soil	C×S	1	0.64	0.23	0.64	**0.08**

The mean value indicates the average of all farms SOC stock (Mgha^{-1}) at a given FD level. C_{grass} and C_{cereal} treatment means of grass and cereal cropping treatments, respectively, across all fine soils (clay) and coarse soils (sand). S_{clay} and C_{sand} are treatment means of fine and coarse soils over all cropping histories, respectively. Their differences represents main effects of cropping and soil texture. Interaction effect ($C_{grass}S_{clay}$-$C_{cereal}S_{clay}$)-($C_{grass}S_{sand}$-$C_{cereal}S_{sand}$) shows how much larger the differences of grass and cereal cropping is in fine soils compared with course soils.

Figure 15 shows that all the treatments were statistically non-significant at the ESM level of 0-2000 Mgha^{-1}, 2000-4000 Mgha^{-1} and 4000-6000 Mgha^{-1}. However, at 6000-8000 Mgha^{-1}, the treatments were statistically significantly different. The treatment coarse soils cropped with cereal were significantly different from fine soils cropped with grass. The treatments coarse soil cropped with grass and fine soils cropped with cereal showed a similar effect.

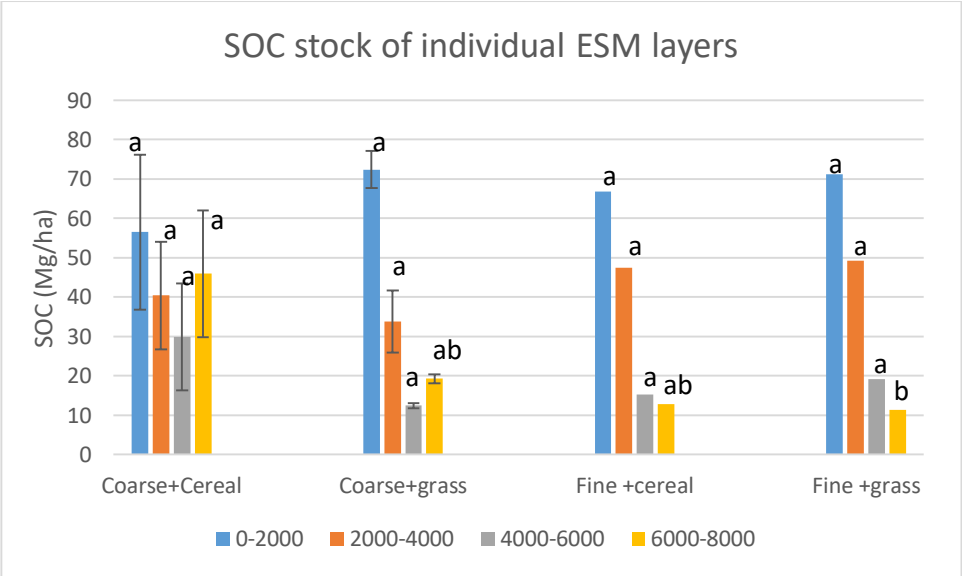


Figure 15. Distribution of soil organic carbon (SOC) (Mgha^{-1}) as related to individual ESM layers in different experimental treatments. Significant differences among different ESM layers in a given treatment are denoted by different lower-case letters.

The results compiled in the Table 5 were estimated based on equivalent soil mass method. The mean value as the farm average of different soil profiles indicated numerically the highest SOC status at 0-2000 Mgha^{-1} compared to all the other individual layers. Even if the value was not statistically significant by ANOVA, there was a tendency of fine textured soils (clay soils) to have larger SOC stocks than coarse textured soils (sandy soils) in the upper layer, but unexpectedly smaller stocks at the lower depths (above 4000 Mgha-^{1}, significant at 6000-8000 Mgha^{-1}). The layer wise SOC stocks showed a decreasing trend towards deeper layers, with broadly similar values in the ESM layers 4000-6000 and 6000-8000 Mgha^{-1}. The highest amount of SOC was surprisingly observed in the deepest layers of sandy soils cropped with cereals, rather than clay soil cropped with grass (Table 5).

Table 5. Effects of experimental factors crop (C) and soil (S) and their interaction (C×S) on SOC stock in the middle layers of ESM soil mass.

Equivalent soil mass layers ($Mgha^{-1}$)

SOC stock ($Mgha^{-1}$)

			0-2000[4]	**2000-4000**	**4000-6000**	**6000-8000**
Effect		**Mean:**	**66.8**	**42.8**	**19.2**	**22.4**
C_{grass}-C_{cereal}			10.2	-2.4	-6.9	-14.0
S_{clay}-S_{sand}			4.62	11.3	-3.9	-20.5
($C_{grass}S_{clay}$-$C_{cereal}S_{clay}$)-($C_{grass}S_{sand}$-$C_{cereal}S_{sand}$)			-11.5	8.4	21.3	25.2
	MS_{error}		421.04	290.33	150.38	297.20
	n		3	3	(2..) 3	(1..) 3
	SEM		11.85	9.84	7.08	9.96
		df		**p level**		
Crop	C	1	0.42	0.82	0.37	0.23
Soil	S	1	0.70	0.29	0.59	**0.09**
Crop× Soil	C×S	1	0.64	0.68	0.17	0.28

The mean value indicates the average of all farms SOC stock ($Mgha^{-1}$) at a given ESM level. C_{grass} and C_{cereal} treatment means of grass and cereal cropping treatments, respectively, across all fine soils (clay) and coarse soils (sand). S_{clay} and C_{sand} are treatment means of fine and coarse soils over all cropping histories, respectively. Their differences represents main effects of cropping and soil texture. Interaction effect ($C_{grass}S_{clay}$-$C_{cereal}S_{clay}$)-($C_{grass}S_{sand}$-$C_{cereal}S_{sand}$) shows how much larger the differences of grass and cereal cropping is in fine soils compared with course soils.

5.3 Comparison of SOC stock estimation by fixed depth (FD) and equivalent soil mass (ESM) methods

5.3.1 Paired T-test for comparing ESM and FD methods in topsoil at ESM 2000 $Mgha^{-1}$

A paired T-test was conducted with the treatment means (farm means, n =12) to explore the significant differences in SOC estimation between FD and ESM methods. The test was carried out by comparing the SOC estimates obtained by ESM method at ESM 2000 $Mgha^{-1}$ to the estimates obtained by using the ESM depth (= the soil depth corresponding to the given ESM level, in this case 2000 $Mgha^{-1}$) in the calculation by FD method (Table 6). The test showed that the SOC stock estimates in the topsoil corresponding to ESM 2000 $Mgha^{-1}$ by the different methods did not differ. Both methods gave a similar estimates of 67 Mg (SOC) ha^{-1}. The SOC stocks estimated by FD method

[4] ESM layer (0-2000) is also repetition of table 3 and mentioned for similar reason.

was numerically very close to those given by ESM method (mean difference less than < 0.5%). Thus, the result supports the view that the ESM depths were accurate and the difference in the estimation error caused by polyline interpolation in these methods was insignificantly small (Table 6).

Table 6. Paired T-test for comparison of SOC stock estimation by ESM method at 2000 $Mgha^{-1}$ soil mass by using the ESM depth in FD method.

Methods	N	Mean	SD	SEM	t	df	p
FD	12	67.07	19.53	5.64	0.47	11	0.65
ESM	12	66.77	18.68	5.40			

N Number of sample, SD standard deviation, SE standard error of mean, df degrees of freedom, p risk of rejection error

The mean ESM depths corresponding to ESM 2000 $Mgha^{-1}$ and used for FD estimation ranged between 18 and 22 cm (Table 7). The variation in the SOC stock estimates was quite clearly higher in coarse soils compared to fine soils. The values of coefficients of variation ranged between 12-60% without any clear difference in the variability of estimates by FD and ESM methods. Both the ESM method and FD method using ESM depth gave usually very similar SOC stocks estimates irrespective of treatment. However, in coarse soil cropped with grass, the FD method gave about 5% higher estimates compared with ESM methods. Even the largest difference between methods was much smaller than the variation in the data (Table 7).

Table 7. Mean SOC stock ($Mgha^{-1}$) (Mean ± SE) and standard deviation (SD) (2000 $Mgha^{-1}$).

	FD based on ESM depth				ESM soil mass (2000$Mgha^{-1}$)		
Treatments	Mean ± SE	SD	CV%	ESM depth	Mean ± SE	SD	CV%
Coarse+cereal	56.5±19.6	34.0	60.2	18.1	56.5 ± 19.6	33.9	60.1
Coarse +grass	76.8±12.4	22.2	28.8	21.1	72.4 ± 11.2	19.3	26.7
Fine +cereal	66.0± 5.4	9.1	13.8	20.3	66.8 ± 4.7	8.2	12.3
Fine+ grass	70.9± 5.5	9.3	13.0	21.7	71.3 ± 5.5	9.5	13.3

SE standard error, SD standard deviation, CV coefficients of variation

5.3.2 Paired T-test for comparing ESM and FD methods at 4000 Mgha^{-1}

A paired T-test for comparison of ESM and FD at ESM 4000 Mgha^{-1} (upper part of subsoil, just below the topsoil horizon) was conducted in the same way as explained above for ESM 2000 Mgha^{-1}. The SOC stock by FD method was calculated using the equivalent depth corresponding to ESM 4000 Mgha^{-1}. The result from this test also confirmed that the estimation by the different methods did not differ significantly (Table 8).

Table 8. Paired T-test for 4000 Mg/ha and its ESM depth for FD and ESM method comparison, N (Number of sample), M (Mean), SD (Standard deviation), and SEM (Standard error of mean).

Methods	N	Mean	SD	SEM	t	df	p
FD	12	109.23	31.65	9.14	-0.50	11	0.62
ESM	12	109.51	30.21	8.72			

N Number of samples, SD standard deviation and SEM standard error of mean

The variation in the SOC stock estimates in all treatments were closely similar, except for clearly higher in coarse soils under cereals (Table 9). The values of coefficients of variability ranged between 17-64% without any clear difference in the variability of estimates by FD and ESM method. Also in this test both the ESM method and the FD method using the ESM depth gave usually very similar SOC stock estimates irrespective of treatment. The largest mean difference was observed in coarse soil cropped with cereals, where the FD method gave about 2% higher estimates compared with ESM method (Table 9). This was much smaller than the variation in the data.

Table 9. Method-wise comparison of mean SOC stocks in different treatments.

Treatments	FD based on ESM depth			ESM depth[5]	ESM, soil mass (4000 Mgha^{-1})		
	Mean± SE	SD	CV%		Mean± SE	SD	CV%
Coarse+cereal	94.7±34.9	60.5	63.9	31.3	96.9 ± 33.2	57.6	59.4
Coarse +grass	106.5±10.4	18.1	16.9	34.8	106.5±10.3	17.8	16.8
Fine + cereal	114.8±12.9	22.4	19.5	34.4	114.4±12.6	21.7	19.1
Fine+ grass	120.9±12.1	20.9	17.3	37.3	120.6±12.1	20.9	17.4

6 Discussion

6.1 Methodological considerations about FD and ESM methods

The estimation of soil organic carbon (SOC) stock was assessed by both fixed depth (FD) and equivalent soil mass (ESM) methods. The comparison was drawn at 2000 and 4000 $Mgha^{-1}$ based on the given equivalent soil mass depth. The difference between the methods for the estimation of organic carbon stock was not significant though there were numerically a slight increment observed in FD method as observed in Table 7 and 9. It was observed that the estimated values in different experimental groups varied only very slightly by the ESM method and the FD method. It was also revealed that in some cases, the estimation tended to be slightly higher by the FD method, and sometimes by the ESM method. This minor difference of estimation could be due to some measurement errors during the estimation. Besides, it might have been originated from the use of the polyline interpolation of measured data to get the estimates at a given FD and ESM levels, as its accuracy might have been slightly different between the two methods. The cubic spline interpolation used in many other studies probably was more accurate (Wendt and Hauser 2013). The difference of SOC estimation in both the methods was within a few percent in this study based on a given ESM depth and the variability of estimates was also broadly similar indicating an insignificantly small difference between the two methods. This highly close SOC estimation by both the methods confirmed that the calculation procedures of each method are reliable. Usually, however, FD method is used for some typical fixed depth layers such as (0-10, 0-20 cm depths) without previous data for selecting an appropriate sampling depth. In this case, the difference of SOC estimation by the FD and ESM method can generally be expected even by a large amount (Hu et al. 2016).

It is well established that the difference between FD and ESM methods is related to the variation of bulk density in soil profile as caused, for instance by soil compaction. As opposed to ESM method, FD estimation is subject to any change in soil bulk density that is needed for measuring SOC stock (Wendt and Hauser 2013). Such an expected impact of the variation in bulk density was also illustrated in an individual field in our study. The experimental "Ja" farm showed that the soil compaction varied SOC content in fixed depth assessment in all the replicates especially in topsoil while the estimated SOC amount by ESM method were less varied in almost all depths and replicates (Figure 11). This result is harmonious with the finding of the previous study (Lee et al. 2009) where they found a larger estimated SOC stocks due to differences in bulk density as caused by soil compaction.

[5] ESM depth is equivalent soil mass depth to which FD method is applied.

However, with a few exceptions, the differences between FD and ESM methods were not significantly observed when considering the larger data set in this study instead found broadly similar. The underlying reasons for the unexpected findings of no difference are not fully clear. Possibly, one important factor could be the soil compaction in the sampling tube that caused during sampling. The partial filling of tubes were commonly observed on sampling and explained part of the increase in bulk density as evident by the significant linear regression model. The resulting soil compaction probably obscured some of the differences in original soil bulk density profiles in the different treatments and so did between the two methods.

Besides, the unequal replicate numbers at greater soil depth resulting from the technical problems seemed to make the estimates less precise and also the comparison of treatments more difficult. In addition, the spatial and temporal variability of data due to bulk density may have decreased the relative importance of method selection for SOC estimates and comparing the treatments in this study. Hence, the soil carbon concentration data could be reliably used for comparison instead of estimating soil carbon stock (Amador et al. 2000; Lee et al. 2009).

6.2 Effects of soil textures on soil organic carbon stock

In this study, the layer wise and cumulative soil SOC data with soil depth was analyzed by FD method at 20 cm depth intervals to the depth of 80-cm and by the equivalent soil mass (ESM) method at 2000 $Mgha^{-1}$ ESM intervals up to the ESM of 8000 $Mgha^{-1}$. The effect of soil texture on SOC stock was not significant in almost any of the layers. However, according to the FD method, the SOC stock was higher in coarse soils than in fine textured soils at the depth layer of 40-60 cm and 60-80cm (Table 4) and the later one was statistically significant. The similar result was observed in case of ESM method as well. The contrasting result from the expected fine soils with higher SOC stock was probably evolved from the presence of some untypical soil horizons. The higher SOC stock in coarse soils could be resulted from the effect of underlying buried organic materials. Besides, there might be presence of easily translocated labile organic carbon resulted from the upper layers that contributed to higher SOC stock for this coarse soils at this depth.

On the other hand, in different layers especially at 20 cm and 40 cm depth, the effect of clay soils on increasing SOC stock was seemingly found to be greater that of coarse soils. The similar high effect of clay soils of SOC accumulation was observed at 2000 $Mgha^{-1}$, 4000 $Mgha^{-1}$ and 6000 $Mgha^{-1}$ ESM layers. This apparently increased SOC stock was probably held due to the reduced decomposition of soil organic matter (SOM) as it decreases with the increase in depth (Hogg 1993). Similarly it has been revealed from the interaction by our study that, fine textured soils in both cereal and grass

cropping seem to have a higher tendency in increasing amount of SOC stock than that of coarse textured soils (Table 2 & 4) and it has been found in all the layers with few exceptions especially at the top soil layers. This was estimated and proved by both the methods. Since, clay has the ability to form clay humic complexes and due to their high specific surface area and interlayer SOC retention abilities (Baldock & Skjemstad 2000), the fine textured soil has the better potential for SOC accumulation. Besides, the insignificantly high carbon content in fine mineral soils is probably the result of reduced microbial oxidation of SOM due to improved soil aggregation and formation of organo-mineral complexes (Jobbágy & Jackson, 2000). Consequently, in contrast to sandy soils, organic carbon in high clay soils is not easily available for microbes due to chemical adsorption of C onto mineral particles which is physically incorporated within microaggregates (Sissoko & Kpomblekou 2010).

To sum up, in relation to coarse textured soils, the fine textured soil cropped with grass and cereal cropping was appeared to accumulate more organic carbon. Hence, our study is consistent with other previous study (Bird et al. 2003) report that the clay rich soils have the higher SOC storage capacity than sand dominated soils in a 1 meter soil profile.

6.3 Effect of crop type on SOC stock in the soil profiles

In the top-soil layer of (0-20cm) and ESM 2000 $Mgha^{-1}$, though not significant, both fixed depth (FD) and equivalent soil mass (ESM) suggested that the SOC stock was higher in coarse textured soil cropped with grass compared with other treatments (Figure 12 & Figure 13). This is tentatively in line with the findings of Heidmann et al. (2002) who reported a large storage of SOC in sand dominated soils with frequent grass cultivation at the topsoil. In addition, irrespective of soil textures, grass cropping seemed to increase the SOC stock in comparison with cereal cropping practices in the experimental farms during 2014 to 2018. The potential reason for high SOC stock in the topsoil could be due to manure application and soil organic amendment. Besides, the grass cropping in coarse textured soil probably increased SOC because of its extended vegetation period.

The estimated amount of SOC was observed seemingly higher under grass cropping than cereal cropping practices. Nevertheless, in some cases especially in the deeper soil layers of different FD and ESM layers, the mean SOC stock was lower under grass cropping than cereals. The contribution of grass and cereal cropping to SOC at 60-80 cm depth was found statistically significant. The cereal cropping unexpectedly showed a higher effect on SOC stock compared to grass cropping (Table 4 & 5). The reason could be due to buried organic horizon that was covered in the upper soils by transported materials. The underlying organic materials that were buried in the deep soil layers could

potentially result in higher SOC accumulation (Appendix 1 & 2). In separate studies by Harrison et al. (2011) and Chaopricha & Marín-Spiotta (2013) found 30-75% of soil carbon of the total carbon stock in the soils below 30cm when the study focused on sandy loam, silt loam of various volcanic, alluvial, colluvium and aeolian deposits that happened to deposit over organic horizon and surface mineral soils.

However, in most of the cumulative soil layers particularly throughout the ESM layers of 2000 $Mgha^{-1}$ to 8000 $Mgha^{-1}$, grass cropping tended to increase SOC stock more than cereal cropping did, but the layer wise ESM data showed that this non-significant trend was limited to only the topsoil layer (Table 3 & 5). The amount of SOC was also observed higher in some FD levels particularly at 0-60 cm. It was revealed that in most cases grass production tended to increase the SOC stock in the examined farms compared to cereal cultivation. Such effects are also as expected due to increased organic matter input to soil by high root biomass and extended rooting depth that influences the decomposition of organic matter (Daly et al. 2000). It is usually considered that the deep layers' stable and recalcitrant SOC forms are especially slow to degrade (Batjes 1996). Besides, high lignin contents might increase the recalcitrance of SOC stock and slow down decomposition process (Melillo et al 1989). In addition, the formation of macroaggregates is higher and rapid due to high residue quality with lower C/N ratio which contribute to the trapping of SOC. In our study, such non-significant tentative effects were seen only in some soil layers. Therefore, our study is partly consistent, for instance, with previous studies reporting that the perennial forage grass cultivation promoted the SOC storage compared with maize and other annual crops (Li et al. 2018).

Besides, the interaction between soil and crop was found significant by FD method at the cumulative depth level of 80 cm. The difference of SOC stock between grass and cereal cropping in fine soils was the higher than that of coarse soils at this depth. Similarly, the combination of grass cropping in clay soils seemingly increased the organic carbon at 40 cm and 60 cm depth compared to coarse soils (Table 2). The SOC stock particularly in the deeper layers including ESM 6000 $Mgha^{-1}$ and 8000 $Mgha^{-1}$ tended to be high probably due to higher belowground storage and lower microbial activity. This was evident in a previous study (Baer et al. 2010) where they found that the silt and clay dominated soils with a history of grass cultivation contributed to the larger amount of SOC in the soils than the sand or coarse textured soil sites. It can be explained that the improved soil structure and aggregation process along with root litter deposition has contributed to higher amount of SOC by increasing protection of soil organic matter (SOM). Besides, this tentatively high amount of SOC held in grass cropping farms was probably stored and protected as mineral associated organic matter which is more persistent. This prediction of organic carbon storage in mineral associated organic

matter (MAOM) is consistent with a previous study (Cotrufu et al. 2019), who reported in their findings that grassland soil organic carbon is predominantly a mineral associated fraction of soil organic carbon.

6.4. Future research needs

The current study has paved the way of further research on deep soil organic carbon and deep soil sampling for SOC sequestration. The future research should be carried out in the deeper soil layers of up to 1 meter or even below 1m depth for better estimation of SOC. In this regard, the limitation of this study was the availability of data in the deeper layers and confining to 80 cm depth of data analysis which might be taken care in the future studies. In our study, from the expected total of 360 soil slices, we processed only 261 soil slices indicating almost 30% of the soil slices were missing due to unparalleled soil column inside the sampling tubes. It probably caused during soil sampling due to technical problems or soil compaction that restricted the tubes to dig down to 1 m depth. Besides, the soil slices below 80 cm depth were not consistently available in all replicate soil tubes that confined the analysis to 80 cm depth. It is considered that an inadequate soil sampling might be incomplete and misled the conclusion on soil total carbon and the capacity of subsoil to sequester carbon. This was emphasized by a previous study that reported the soil sampling that was not deeper than 1.5 m significantly underestimated the soil organic carbon (James et al 2014). In future research, sampling can be done with more sophisticated sampling equipment so that it reaches easily to the desired depth. However, it will also be interesting to analyze our studied soils furthermore to investigate the share of soil inorganic carbon added to the soils by different soil amendments if any. A study conducted by Dong et al. (2019) found that long-term biochar application increased the soil inorganic carbon in different depths. The future research can also take into account within-field spatial variability of soil texture and structure. Based on the results of our current study, it can be recommended that the analyses of consistent amount of data from a larger range of fields representing different soil types with a longer record of cropping history are needed to understand the effect magnitude of SOC level and SOC dynamics. Such considerations might help to make more robust conclusions for the studied soils.

7 Conclusions

The findings of this study described the effect of different soil management factors responsible for influencing the SOC stock in the different farms of Carbon Action Project located in Western and Eastern Finland. The size of estimated soil organic carbon stocks in fine and coarse textured soils under cereal and grass cropping practices did not differ significantly in this study except at 60-80 cm depth by FD method. The amount of SOC under cereal cropping was significantly higher than the amount under grass cropping at this depth level which was probably due to buried organic-rich subsoil horizon. Grass cropping practices in fine textured soils seemed to provide more protection to SOC in some soil layers and hence tentatively increased the storage higher than in cereal treatments. Fixed depth and equivalent soil mass procedures for estimating the SOC content did not show any significant difference for the different experimental treatments in our study even though equivalent soil mass is a method of choice for its high accuracy compared to FD method. Thus, the absolute reasons for distinguishing between the methods can be further studied. However, without temporal monitoring, almost the same conclusions about the effects of treatments and soil depth could be drawn by both methods. From the directly measured soil carbon contents and bulk density in addition to soil depth, it was revealed that the bulk density increased with increasing soil depth while it decreased with increasing organic carbon content, as expected based on previous research and also by partial filling of soil tubes' on sampling. In this regard, the consequences of these factors for the SOC estimation by different methods should be studied for further clarification in future research.

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10 Appendices

Appendix 1: SOC content (% by weight) of individual soil samples from different treatments at different soil depths **Soil depth/cm**

Sample id	Tube code	Treatments (Soil texture +crop type)		0-10	10-20	20-30	30-40	40-50	50-60	60-70	70-80	80-90	90-100
1	Mä	Fine	Cereal	4.14	3.11	2.57	0.77	0.91					
2	Pu	Fine	Cereal	3.50	3.17	2.56	0.82	0.73	0.68	0.64			
3	Ja	Fine	Cereal	3.31	2.96	3.13	1.33						
4	Mä	Fine	Cereal	3.61	3.54	2.11	1.00						
5	Ja	Fine	Cereal	3.41	2.94	2.94	1.30	0.73	0.67	0.66	0.61	0.59	0.59
6	Pu	Fine	Cereal	3.49	3.57	3.03	0.99	0.72	0.66	0.62	0.64		
7	Mä	Fine	Cereal	4.51	3.90	4.93	1.78	1.10					
8	Ja	Fine	Cereal	2.90	2.85	1.77	0.84	0.81	0.66	0.67	0.63	0.64	
9	Pu	Fine	Cereal	2.94	2.76	2.70	0.52	0.45	0.37	0.32	0.31		
10	Kp	Fine	Grass	3.20	2.92	2.53	1.00	0.75	0.65	0.61			
11	Pa	Fine	Grass	3.65	3.23	2.75	0.94	0.48	0.40	0.31	0.29	0.28	0.24
12	Ke	Fine	Grass	4.39	4.25	**4.61**	**4.87**	1.81	0.63	0.63	0.70	0.31	0.28
13	Kp	Fine	Grass	2.97	3.17	2.17	0.86	0.71	0.62	0.66	0.52		
14	Pa	Fine	Grass	3.89	4.08	3.24	1.24	0.68	0.51	0.54			
15	Ke	Fine	Grass	3.84	3.71	**4.16**	1.55	0.51	0.38	0.35	0.28	0.28	
16	Kp	Fine	Grass	3.16	3.01	1.12	0.73	0.67	0.58	0.57	0.64		
17	Pa	Fine	Grass	3.73	6.96	5.15	2.07	0.92	1.35	0.58	0.58	0.52	0.53
18	Ke	Fine	Grass	2.94	2.57	2.48	2.27	0.77	0.68	0.61	0.59	0.55	0.63
19	Ik	Coarse	Cereal	4.65	4.22	4.60	0.73	0.72	0.85	1.01	0.99		
20	Ru	Coarse	Cereal	2.93	2.89	2.38	0.69	0.62					
21	Ko	Coarse	Cereal	1.29	1.15	0.86	0.52	0.51	0.51				
22	Ik	Coarse	Cereal	4.97	4.90	3.96	1.55	0.56	0.69				
23	Ru	Coarse	Cereal	2.63	2.96	2.53	0.82	0.71	0.79				
24	Ko	Coarse	Cereal	1.49	1.46	1.20	0.60						
25	Ik	Coarse	Cereal	*20.23*	*21.91*	*17.91*	*1.78*	*0.71*	*0.70*	*0.67*	*1.03*		
26	Ru	Coarse	Cereal	2.47	1.43	0.78	0.71	**3.47**	3.02				
27	Ko	Coarse	Cereal	1.37	0.55	0.55	**6.00**	**4.96**					
28	ML	Coarse	Grass	3.63	4.44	1.21	0.51	0.51	0.49	1.52	0.48		
29	Ni	Coarse	Grass	2.95	2.75	1.48	0.39	0.35					
30	Jo	Coarse	Grass	2.20	0.96	0.66	0.72	0.62	0.63	0.62	1.69	1.51	
31	ML	Coarse	Grass	4.04	4.24	1.32	0.28	0.22	0.17	0.15	0.16		
32	Ni	Coarse	Grass	*19.67*	*19.80*	*30.41*	*10.15*	*0.19*					
33	Jo	Coarse	Grass	3.44	3.02	3.15	2.09	0.73	0.64	0.57	0.60	0.54	
34	ML	Coarse	Grass	5.32	10.90	1.00	1.14	1.13	0.48	0.48	0.47		
35	Ni	Coarse	Grass	5.55	5.35	3.68	0.64	0.56	2.66	2.87			
36	Jo	Coarse	Grass	2.75	2.83	2.24	1.02	0.64	0.59	0.60	0.57		

Organic soils excluded from this study are shown with an italic font and mineral subsoil layers with SOC contents higher than those in topmost soil layers are shown with a bold font.

Appendix 2: Bulk density (% by weight) of individual soil samples from different treatments at different soil depths **Soil depth/cm**

Sample id	Tube code	Treatments (Soil texture +crop type)		0-10	10-20	20-30	30-40	40-50	50-60	60-70	70-80	80-90	90-100
1	Mä	Fine	Cereal	1.11	1.42	1.52	1.16	1.30					
2	Pu	Fine	Cereal	0.66	1.16	1.45	**1.70**	1.65	1.69	1.56			
3	Ja	Fine	Cereal	0.72	1.00	1.16	1.46						
4	Mä	Fine	Cereal	1.23	1.45	1.44	1.32						
5	Ja	Fine	Cereal	0.60	0.85	1.15	1.42	1.49	1.48	1.45	1.44	1.43	1.16
6	Pu	Fine	Cereal	0.67	1.09	1.40	**1.74**	1.69	1.61	1.61	1.58		
7	Mä	Fine	Cereal	0.79	1.38	1.35	1.41	1.30					
8	Ja	Fine	Cereal	1.13	1.31	1.36	1.41	1.45	1.46	1.48	1.44	1.45	
9	Pu	Fine	Cereal	0.66	1.04	1.35	1.67	1.69	1.70	1.67	1.61		
10	Kp	Fine	Grass	0.93	1.45	1.57	1.59	1.37	1.36	1.19			
11	Pa	Fine	Grass	0.59	0.93	1.20	1.50	1.47	1.46	1.49	1.46	1.42	1.44
12	Ke	Fine	Grass	0.66	1.01	1.12	1.28	1.27	1.19	1.41	1.47	1.44	1.40
13	Kp	Fine	Grass	0.75	1.11	1.51	1.54	1.54	1.48	1.43	1.41		
14	Pa	Fine	Grass	0.56	0.96	1.32	1.65	1.68	1.70	1.69			
15	Ke	Fine	Grass	1.02	1.03	1.18	1.41	1.49	1.54	1.52	1.48	1.51	
16	Kp	Fine	Grass	0.87	1.42	1.63	1.53	1.46	1.51	1.38	1.21		
17	Pa	Fine	Grass	0.86	0.58	0.74	1.50	1.48	0.76	1.46	1.48	1.53	1.50
18	Ke	Fine	Grass	0.86	1.13	1.33	1.37	**1.71**	1.54	1.47	1.43	1.34	1.17
19	Ik	Coarse	Cereal	1.08	1.11	1.40	**1.77**	**1.71**	1.56	1.50	1.49		
20	Ru	Coarse	Cereal	0.96	1.35	1.59	**1.81**	**1.74**					
21	Ko	Coarse	Cereal	1.23	1.60	**1.76**	**1.82**	**1.79**	**1.75**				
22	Ik	Coarse	Cereal	0.71	1.05	1.28	1.69	1.59	**1.70**				
23	Ru	Coarse	Cereal	0.73	1.28	1.50	1.54	1.70	1.45				
24	Ko	Coarse	Cereal	0.98	1.48	1.61	1.65						
25	Ik	Coarse	Cereal	*0.35*	*0.51*	*0.68*	***1.85***	***1.76***	***1.73***	*1.52*	*1.48*		
26	Ru	Coarse	Cereal	1.02	1.53	1.40	**1.74**	**1.87**	**2.38**				
27	Ko	Coarse	Cereal	1.14	1.55	1.62	**1.83**	**1.77**					
28	ML	Coarse	Grass	0.77	1.05	**1.70**	**1.86**	**1.73**	1.66	0.54	1.63		
29	Ni	Coarse	Grass	0.76	1.26	**1.72**	**1.82**	**1.83**					
30	Jo	Coarse	Grass	0.87	1.27	1.38	1.28	1.29	1.32	1.26	1.30	1.23	
31	ML	Coarse	Grass	0.83	1.08	**1.78**	**1.77**	**1.72**	**1.72**	1.69	1.66		
32	Ni	Coarse	Grass	*0.35*	*0.53*	*0.43*	*0.78*	***1.94***					
33	Jo	Coarse	Grass	0.82	1.03	1.23	1.34	1.26	1.29	1.34	1.31	1.41	
34	ML	Coarse	Grass	0.65	0.49	1.68	0.87	0.79	**1.71**	1.57	1.68		
35	Ni	Coarse	Grass	0.72	1.06	1.40	**1.82**	**1.76**	1.58	1.49			
36	Jo	Coarse	Grass	1.10	1.29	1.53	1.53	1.53	1.38	1.36	1.31		

Organic soils excluded from this study are shown with an italic font.

Sample with notably high BD values above 1.70 Mg m^{-3} are shown with a bold font.